VOLUME V JANUARY, 1913 No. 4

THE FIXATION OF RABIES VIRUS IN THE MONKEY (*MACACUS RHESUS*) WITH A STUDY OF THE APPEARANCE OF NEGRI BODIES IN THE DIFFERENT PASSAGES.

BY CAPTAIN HUGH W. ACTON, I.M.S.,
Assistant Director,

AND MAJOR W. F. HARVEY, I.M.S.,
Director, Pasteur Institute of India, Kasauli.

(With Plate X.)

PASTEUR, in May 1884, showed that if the rabies virus of the dog was passed from rabbit to rabbit, or guinea-pig to guinea-pig, it gradually became exalted in virulence and fixed in incubation period for these rodents. This fixed virus remained exalted in virulence when inoculated subdurally into other animals. Magendie held the opinion that the rabies virus, when transmitted by bites from dog to dog, lost its virulence by about the fifth passage.

Celli and Marino-Zucco (1892) held identical views, and considered them confirmed by their experiments on dogs. They found that from the 6th to the 10th passage (employing the subdural or intra-ocular mode of infection) their dogs no longer showed furious rabies, but died from the dumb or paralytic form. As they continued these passages, their dogs now began to die of a curious consumptive or cachectic type of rabies which developed after a very long incubation period. The brains of these dogs suffering from this chronic type of rabies were no longer capable of transmitting the disease to rabbits—*i.e.* had lost all virulence.

Marie (1907) and Lamb and McKendrick (1909) showed, however, that this was not the case for dogs, but that street virus became fixed in this animal, just as it did in the rabbit. In a recent experiment, on the passage of fixed virus of the rabbit through the dog, we found

that the virus remained fixed for the dog, but in the 5th passage, the animal did not show rabies, that is to say complete failure to infect had occurred. But when this same dog was reinoculated 20 days later from the glycerine preserved brain of passage dog 4, it contracted dumb rabies after the fixed virus incubation period, thus showing that the loss of the virus was not due to attenuation, but to technique.

TABLE I. *Showing the passage of fixed virus in the dog.*

No. of passage	Wt. of dog in grammes	Incubation period: death	Cultures from brain	Type of rabies
1st	10,000	8/10	Negative	Paralytic
2nd	11,060	6/7	,,	,,
3rd	11,500	7/9	,,	,,
4th	10,640	7/10	,,	,,
5th	8,350	*a*, Escaped to subdural inoculation		*a*, Escaped rabies
		b, Reinoculated 8/11	*b*, Negative	*b*, Paralytic rabies

Escape to the subdural test has been largely taken by writers on the subject of rabies immunization to be synonymous with immunity in the test animals. In a memoir which is in preparation we shall deal with this subject at length, and show how faulty technique, refluxes of the virus, and contact with antiseptics are all factors which contribute to escapes. Therefore we hold that in all experimental work on rabies the controls should be equal in every respect (*i.e.* in number, size etc.) to the test animals. We regard many of the contrary opinions explicable simply on the grounds of faulty technique and consider that this error would be eliminated if proper attention were paid to controls.

At the International Congress at Copenhagen, Pasteur (1884) stated that "On the 6th of December 1883, the medulla of a rabid dog, in which rabies had been induced by the inoculation of the virus of a child dead of rabies, was inoculated into a monkey by trephining. The monkey developed rabies eleven days later; from this first monkey an inoculation was made into a second, which still took rabies on the 11th day. In a third monkey rabies did not show until the 23rd day, etc. The medulla of each of these monkeys was inoculated by trephining into two rabbits for each passage. The rabbits inoculated from the first monkey took rabies on the thirteenth and sixteenth day, respectively; those from the fourth on the 28th day; those from the fifth on the 27th day; those from the sixth on the 30th day." Pasteur did not state the species of monkey used.

In *Macacus rhesus*, we found the street virus of the dog became exalted and fixed in this animal just as in the rabbit and guinea-pig (Pasteur, 1884); the dog (Marie, 1907; Lamb and McKendrick, 1909); the cat (Blasi and Russo-Travali, 1894) etc.

In the first two passages in this species of monkey, the rabies was clinically of the furious type; from the 3rd to the 6th passage, the symptoms commenced with fury and for the last 24 to 48 hours the animals were completely paralysed; from the 7th passage onwards the paralytic type alone was observed. The virus we used was derived from a dog but had undergone one passage in a rabbit. Negri bodies were seen both in the dog's brain and in the rabbit's brain; cultures from the brain, heart blood and liver of the rabbit were sterile. The incubation period in the rabbit was a somewhat short one (10 days), but on the other hand the first monkey inoculated showed an incubation period of 13 days. The virus used by Pasteur produced rabies in the first passage monkey in 11 days.

In the monkey from the 4th passage onwards the incubation period fluctuated between seven and eight days—it is difficult to determine the earliest symptoms in the monkey. The incubation period of the rabbits inoculated from the monkeys showed a fixed virus incubation period from the 7th passage onwards. Table II gives the

TABLE II.

Street virus passage in Macacus rhesus. *The virus was obtained from a dog suspected of rabies, and sent from Burma. Negri bodies were found in the dog's brain: the rabbit inoculated from it showed signs of rabies on the 10th day and died on the 13th. Cultures from the brain, liver and heart's blood were sterile; Negri bodies were found in the rabbit's Hippocampus major.*

No. of passage	Weight of monkey in grammes	Incub. period / Death	Negri bodies	Cultures	Type of rabies	Rabbit Incub. period / Death	Dog Incub. period / Death
1st	3850	13/14	+	–	Furious	—	—
2nd	4000	10/11	+	–	,,	—	—
3rd	1920	9/12	+	–	Mixed	9/12	—
4th	2300	7/9	+	–	,,	8/11	—
5th	3630	7/9	+	–	,,	8/12	—
6th	3650	8/11	+	–	,,	8/10	—
7th	5200	7/10	+	–	Paralytic	7/11	—
8th	1800	8/12	+	–	,,	7/10	—
9th	3500	7/12	+	–	,,	7/10	—
10th	2050	8/11	–	–	,,	7/11	—
11th	2000	8/11	–	–	,,	7/10	—
12th	3000	7/10	–	–	,,	7/11	8/10

details of these twelve passages in *Macacus rhesus*, this number of passages being considered sufficient to establish our contentions.

A converse experiment was also conducted to show that the fixed virus of the rabbit, when passed through *Macacus rhesus*, is not altered in its virulence either for this monkey or for the rabbit.

Table III illustrates this point:—

TABLE III.

The virus used was the ordinary fixed virus of the passage series and obtained from passage rabbit No. 92 *of the* 15/8/11.

No. of the passage	Weight of monkey in grammes	Incub. / Death period	Cultures	Rabbit Incub. / Death period
1st	3540	6/10	–	—
2nd	2950	6/9	–	—
3rd	3000	6/12	–	—
4th	1950	7/9	–	7/11

Not continued further as the point was regarded as sufficiently proved.

Pasteur (1884) at this conference went on to state:—"It cannot be doubted that by passage from monkey to monkey and from the different monkeys to the rabbit, the virulence diminishes for these latter animals. It is equally diminished for the dog. The dog inoculated from the medulla of the fifth monkey had an incubation period of not less than 58 days although the inoculation had been performed by the method of trephining. Other observations of the same nature made on a series of monkeys have led to results of the same kind. We are then in possession of a method which permits of attenuation of the virulence of rabic materials. Successive inoculations from monkey to monkey give to the virus, when transferred to rabbits, a power of communicating rabies with a progressively lengthening incubation period. Nevertheless if we start from any one of these rabbits to inoculate successively other rabbits, the production of rabies in them obeys that law of exaltation of the virulence which takes place by passage from rabbit to rabbit and of which we have already spoken. The application of these facts places in our hands a method of vaccinating dogs against rabies."

Our results in the species of monkey which we used are such as to lead us to negative the idea of there being any exception in this mammal, of the law of exaltation of virulence of street virus by subpassage, as far as it relates to subdural inoculation.

The formation of Negri Bodies.

In a previous memoir (1911) we may not have made it sufficiently clear that we considered that the granules, which we described as the commencing stage of the Negri body, were those described by some authors as the specific parasites of rabies, which, by the development of a veil around them, produced the Negri body. The granules regarded by us as consisting of nucleolar matter are of all sizes ranging from almost ultra-microscopic particles up to those of 1–2μ in diameter. We take this opportunity of making that meaning clear. We were further guilty of using the term "Chlamydozoa" rather loosely by seeming to make it include the older genera *Neuroryctes*, and *Cytoryctes*, but we intended it to apply, as far as the contentions in our paper went, to the description quoted from Hartmann on page 256 of our paper. We take this opportunity of thanking the author of the note appended to our article for pointing out our inaccuracy.

TABLE IV.

No. of passage in monkey	Size of Negri bodies											Mean size in μ	Total Negris in 100 cells	Staining reaction
	Under 1μ	1μ	1½μ	2μ	2½μ	3μ	3½μ	4μ	5μ	6μ	7μ			
1st	1	8	8	7	11	6	—	8	—	—	1	2·3	50	Brown
2nd	12	23	12	22	2	20	1	13	2	—	1	2·6	108	Brown to black
3rd	20	54	26	22	4	4	—	2	1	—	—	1·4	133	Brown
4th	23	44	26	17	2	4	—	—	—	—	—	1·2	116	Brown & black
5th	60	23	11	7	1	2	—	—	—	—	—	·9	104	,,
6th	97	54	11	10	2	1	—	—	—	—	—	·8	174	Black
7th	2	1	—	—	—	—	—	—	—	—	—	·6	3	,,
8th	2	—	—	—	—	—	—	—	—	—	—	—	2	,,
9th	1	1	—	—	—	—	—	—	—	—	—	—	2	,,
10th	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11th	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12th	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Our results on the study of Negri bodies in the ganglion cells of the Hippocampus major of *Macacus rhesus* (see Table IV) have confirmed us in our view that the so-called Chlamydozoa in rabies are nothing more nor less than a development round nucleolar fragments. The tissues were hardened in Zenker's fluid, washed and passed through graduated alcohols, cleared, embedded in paraffin wax. The mercury

was removed from the sections, which were then stained with Mallory's Iron haematoxylin and Bordeaux Red.

In each monkey, 100 ganglion cells were examined and the size, number, and colouration of the cell inclusions, noted and recorded. The result of this examination is shown in Table IV, which gives their frequency distribution in size etc.

Our results may be summarized as follows:—

1. With subpassage, Negri bodies diminish in size until they become almost ultra-microscopic.

2. Of large size to begin with and staining brown to black, in the above experiment by the 6th passage they stain wholly black and are identical in staining reactions with the nucleolus.

3. There is a very decided drop in the numbers present at the 7th passage. The significance of this is not exactly clear. It is noteworthy, however, that the 7th passage is that in which the paralytic form of rabies is fully developed. In the 6th passage furious symptoms were still marked.

4. The diminution in the number of Negri bodies shown in the 7th passage, is continued in the 8th and following passages.

5. The Negri bodies of the earlier passages (first three) showed a "veil" arrangement. Those of the later passages did not present this appearance and may be described as "naked."

6. We regard the granules found in "veiled" Negri bodies as being composed of unaltered nucleolar substance.

7. The experiment given seems to show that the Negri body, as found in the cytoplasm of a ganglion cell, tends to disappear altogether from that region with the attainment of fixity of the virus. We have inoculated bullocks—the animals showing the largest Negri bodies known to us—one with street virus and another with fixed virus. The street virus brain showed large Negri bodies; the fixed virus brain showed none at all.

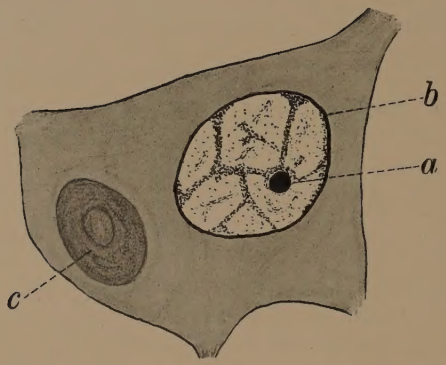


Fig. 1.

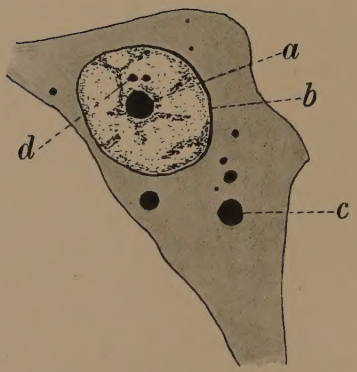


Fig. 2.

REFERENCES.

Acton and Harvey (1911). The Nature and Specificity of Negri Bodies. *Parasitology*, iv, 255–272.

Blasi and Russo-Travali (1894). La rage expérimentale chez le chat. *Ann. Inst. Pasteur*, viii, 338.

Celli and Marino-Zucco (1892). Sulla trasmissione del virus rabico da cane a cane. *Annali dell' Istituto d' Igiene sperimentale d. R. Univ. d. Roma*, ii, 1.

Lamb and McKendrick (1909). Observations on Rabies. *Scientific Memoirs by Officers of the Medical and Sanitary Department of the Government of India.* No. 36.

Marie (1907). L'injection du virus des rues au chien. *Compt. Rend. Soc. Biol.* lxii.

Pasteur (1884). Sur la rage. *Compt. Rend. Ac. Sci.* xcviii, 1229–1231.

EXPLANATION OF PLATE X.

The drawings were made with the aid of a camera lucida, employing eye-piece No. 12, $\frac{1}{12}$″ oil immersion objective and 160 mm. tube extension.

Fig. 1. Ganglion cell from the Hippocampus major of Passage Monkey No. 1. (*a*) nucleolus, (*b*) nucleus, (*c*) brown stained Negri body.

Fig. 2. Ganglion cell from the Hippocampus major of Passage Monkey No. 6. (*a*) nucleolus, (*b*) nucleus, (*c*) Negri bodies of all sizes (iron staining), (*d*) nucleolar fragments inside the nucleus.

THE LIFE-HISTORY OF *DERMACENTOR VARIABILIS*[1].

By SEYMOUR HADWEN, D.V.Sci.,
1st Assistant Pathologist, Dominion Department of Agriculture, Agassiz, B.C.

During the past three years I have made various attempts to raise *Dermacentor variabilis* through its various stages on tame rabbits and have at last been successful. Though there are still many points connected with the life-history to clear up, it is at any rate the first step towards finding ways and means for its control.

To the total of 180·5 days it is necessary to add ten days at each stage for the hardening of the skin, which increases the total to 210·5 days, without taking into account the time occupied in waiting for a host. The period of ten days allowed for the hardening of the skin is about correct for the nymphs, as can be verified by the table. Out of eleven nymphs put on a rabbit on May 9th, only one attached itself, but 13 of those put on May 13th gorged.

Only twelve females and one male were recovered at the end of the experiment, showing that the conditions were adverse to their development. In the first place, the larvae were starved from August 25th, 1911 to February 10th, 1912, a period of five-and-a-half months. Secondly, the temperature at which they were kept was very variable; the nymphal moult being undoubtedly much prolonged on this account. The ticks were kept in unheated rooms the greater part of the time, and the only occasion on which they were kept at a really warm temperature was when they were first put on to the rabbit. On this occasion they had been previously warmed and the cage and room were heated.

In order to form an opinion as to the season of the year in which *D. variabilis* goes through its various stages, it is necessary to say a

[1] *Dermacentor variabilis* (Say, 1821; Banks, 1907) = *Dermacentor electus* (Koch, 1844; Stiles, 1910).

word about Manitoba and the dates on which ticks are most numerous. Most of the gorged females I have obtained were collected in June; the earliest of which I have a record was captured on May 25th, and the latest on July 17th. As soon as the snow disappears, and the warm weather begins, adults are apparently found everywhere and are a great annoyance to both man and beast, but I have no records of larvae and nymphs ever having been taken at this period. This, I believe, is probably due to no systematic search having been made for the immature stages.

Protocol.

			Average No. of days
1 ♀ gorged on a dog. Collected at Aweme, Manitoba, Canada. (N. Criddle)		July 6, 1911	10
Oviposition began		,, 16, ,,	
Eggs hatched		Aug. 26, ,,	41
Larvae put on rabbit		Feb. 10, 1912	
Came off gorged	1 on	,, 13, ,,	$4\frac{1}{2}$
	73 ,,	,, 14, ,,	
	26 ,,	,, 15, ,,	
	20 ,,	,, 16, ,,	
Larvae moulted and nymphs emerged ...	1 on	April 30, 1912	84
	4 ,,	May 3, ,,	
	3 ,,	,, 4, ,,	
	2 ,,	,, 5, ,,	
	10 ,,	,, 7, ,,	
	2 ,,	,, 8, ,,	
	5 ,,	,, 9, ,,	
	4 ,,	,, 10, ,,	
	3 ,,	,, 11, ,,	
	1 ,,	,, 12, ,,	
	5 ,,	,, 13, ,,	
Nymphs put on rabbit; only one attached, and several were recaptured	11 ,,	,, 9, ,,	
Came off gorged	1 ,,	,, 14, ,,	
Nymphs put on rabbit	26 ,,	,, 13, ,,	
Came off gorged	3 ,,	,, 18, ,,	6
	5 ,,	,, 19, ,,	
	4 ,,	,, 20, ,,	
Adults hatched. 12 ♀'s and 1 ♂ ...	1 ,,	June 20, 1912	35
	5 ,,	,, 22, ,,	
	2 ,,	,, 23, ,,	
	3 ,,	,, 27, ,,	
	2 died in act of emerging.		
		Total	$180\frac{1}{2}$

Hosts. Specimens have been taken on man, cattle, horses and dogs. I have no record of other hosts for Manitoba.

Copulation. Copulation has not been observed; presumably it takes place on the host, as about an equal number of males and females seem to occur on the animals, as is the case in other species of *Dermacentor.*

Oviposition. The act of oviposition was observed several times and appears to be similar to that of other species of ticks. One point which seems worthy of note is that no matter how slightly gorged a female is when removed from the host, she will lay a few eggs. The tenacity to life possessed by these partly gorged females is remarkably great as compared to that of fully gorged and ungorged females. This applies also to *D. albipictus* and *D. venustus.*

Larvae. When first put on to a rabbit, the larvae do not seem to feel at home and in every instance many of them have wandered away from this host. In my last experiment I spent fully an hour lifting them back with a brush, at the end of which time no more appeared to be coming off and, as events proved, they remained to gorge. Owing to my early failures I began to think that rabbits were unsuitable animals on which to feed the ticks, especially as I read of other workers having similar difficulties. Accordingly, a number of larvae were placed on a fowl; they at once became extremely active but absolutely refused to remain on the bird.

I do not know on what animals the larval and nymphal stages are generally passed in nature; I can only say that it is possible to raise them on rabbits in the laboratory. During the period when they are not upon the host the immature ticks appear to require moisture. In the laboratory it was found necessary to place pieces of moist filter paper into the tubes in which they were kept.

Nymphs. One peculiar feature I observed about the last moult is that after the gorged nymphs had been put away for a few days, small whitish droplets were seen exuding from their bodies. I at first thought some sort of slimy mould was growing on them and washed it off with clean water, but as it soon reappeared I examined it under the microscope and found that it was a mucoid substance and contained neither bacteria nor moulds. This secretion may serve as a protection against desiccation, especially as the climate of Manitoba is dry. Shortly after the secretion was noticed the skin began to turn white near the capitulum.

Conclusions.

Dermacentor variabilis is a three-host tick and can be raised experimentally on rabbits. Starting with an adult female in the spring, it is very probable that the life-cycle is carried as far as the nymphal moult during the summer and autumn, and that the winter is passed in this state, the adults emerging in the spring. Under laboratory conditions about 210 days are required for adults to issue, reckoned from the time when the gorged mother tick abandoned the host, without taking into account the variable period which the tick may have to wait for a host when unfed.

I am greatly indebted to Mr Norman Criddle, of Treesbank, Manitoba, who has furnished me with all the material, and to the Veterinary Director-General for permission to publish this article.

NEW TREMATODE PARASITES FROM FISHES OF THE ENGLISH CHANNEL.

By WILLIAM NICOLL, M.A., D.Sc., M.D.
(*Lister Institute of Preventive Medicine, London.*)

(With Plate XI.)

During August and September 1909 I had an opportunity, thanks to the Government Grant Committee of the Royal Society, of making a fairly comprehensive examination of the parasites of fishes from the English Channel at the Marine Biological Laboratory, Plymouth. During that time nearly five hundred fishes, belonging to about 80 species, were dealt with and a very extensive collection of parasites made. A considerable number of species were obtained many of which are new to British waters but the number of absolutely new species amounted to very few. Of these I am describing here four of the most noteworthy, three of which appear to be of new generic type. A complete account of the investigations will be published later.

Three of these species were rather uncommon. The fourth was comparatively frequent in the pipe fishes and was found in association with a better known species of the same genus, namely *Podocotyle atomon.*

Podocotyle syngnathi n. sp.

(Figs. 1, 2.)

This species was a not uncommon parasite of the pipe fishes, *Syngnathus acus, Nerophis aequoreus* and *Siphonostoma typhle.* In the first it occurred in four out of nine, in the second in four out of eight and in the third in one out of six. It was not met with in any of the eight specimens of *Nerophis lumbriciformis* examined. Its frequency

in those fishes is therefore nine out of 31, *i.e.* about 29 %. It did not occur in any other species of fish so that it is in all probability a specific parasite of the Lophobranchii. In *Nerophis aequoreus* it was accompanied by *Podocotyle atomon.*

It bears a very close resemblance to the other species of *Podocotyle* but differs from them chiefly in the noticeable shortness of the cirrus pouch.

The specimens varied in length from 2·2 mm. to 5·9 mm. The smallest specimen was obtained from *Nerophis aequoreus* and had just begun to produce ova, so that evidently this species does not attain maturity so early as *P. atomon.* Some mature specimens of the latter measuring little over 1 mm. were obtained from the same fish.

Measurements were made of five of the largest specimens, the average length of which was 5·36 mm. (4·55–5·95 mm.). The greatest breadth (0·78 mm.) occurred usually at the testicular region, but the breadth across the ventral sucker was almost as great and in one case greater. The greatest breadth is therefore about 1/7 of the length. In a fully extended specimen it may be as small as 1/9. The species is therefore more elongated than *P. atomon* but less so than *P. reflexa.* The body is not much flattened and the ventral sucker is usually prominent.

The oral sucker has a diameter of ·38 mm. and the ventral sucker measures 0·43 × 0·55 mm. The latter is situated at a distance of 1·25 mm. from the anterior end, the neck therefore being somewhat more than 1/4 of the body length.

There is a very short prepharynx and the pharynx measures 0·19 mm. in diameter. The oesophagus is about as long as the pharynx (0·17 mm.), and the intestinal diverticula extend nearly to the posterior end of the body becoming slightly dilated during their course.

The excretory vesicle reaches the anterior border of the ovary.

The testes are separated from each other by a short distance (0·21 mm.). They are oval with their long axes slightly tilted from the middle line, the anterior end in both cases being usually directed towards the left side. They overlap the intestinal diverticula only to a very slight extent. Their dimensions are 0·57 × 0·47 mm. Between the posterior testis and the end of the body there is a space a little over 1 mm. (1/5 of the body length, but this varies from 1/6 to 1/4). The genital aperture is situated a little in front of the intestinal bifurcation, decidedly towards the left side. The cirrus pouch is short and rather stout, extending at most not further than the middle of the ventral sucker. The vesicula seminalis is accordingly much more compact than it is in

the other species of the genus. The ductus ejaculatorius is also short.

The ovary lies in front of the anterior testis and is separated from it by a variable space (about 0·1 mm.). It has the trilobate posterior border characteristic of the genus and measures about 0·42 × 0·29 mm. The shell-gland complex resembles that in *P. atomon* but the receptaculum seminis lies rather further forward. The yolk glands are somewhat more limited in extent, stopping a short distance behind the ventral sucker. They are most voluminous posteriorly but are frequently interrupted at the level of the second testis on one or both sides. They do not fill up the inter-testicular space. The transverse yolk ducts unite in front of the ovary. The uterus extends between the receptaculum seminis and the ventral sucker and is confined between the intestinal diverticula. It usually contains about 50 eggs, measuring 0·082–0·102 × 0·045–0·050 mm., the average being 0·092 × 0·047 mm.

Lepidauchen stenostoma n. g., n. sp.

(Fig. 3.)

Only two specimens of this form were found on one occasion in the intestine of *Labrus berggylta*, and it is apparently an uncommon parasite. It is a moderately flat and broad species measuring 2·9–3·25 mm. in length and 1·3 mm. in greatest breadth which occurs about the middle of the body. It tapers slightly towards the ends, which are broadly rounded.

The surface of the anterior part of the body is closely covered with stout spines but these appear to be entirely absent in the posterior part beyond the middle of the body.

The globular oral sucker is subterminal and has a diameter of 0·55 mm. It is characterised by a peculiar aperture which presents the appearance of a longitudinal slit inflated at its anterior end. The ventral sucker is much smaller, measuring only 0·27 mm. in diameter. It is situated at a distance of 1·12 mm. from the anterior end. The neck is thus about 3/8 of the body length.

There is a very small prepharynx followed by a pharynx of comparatively enormous size, its dimensions being 0·31 × 0·34 mm. There is no oesophagus, the intestinal diverticula separating immediately behind the pharynx and passing out at a wide angle towards the sides of the body; they extend almost to the posterior end.

The excretory system was not observed.

The two moderately large globular testes lie in the posterior half of the body, one in front of the other and contiguous. The posterior testis is separated from the end of the body by a space equal to its diameter which is 0·45 mm. The cirrus pouch is small and stout, lying immediately and entirely in front of the ventral sucker. It contains a comparatively large and globular vesicula seminalis but other details of its internal structure were obscured by the presence of eggs in the genital sinus. The genital aperture is in the middle line a little in front of the intestinal bifurcation.

The ovary is situated just in front of, and contiguous with, the anterior testis, but displaced towards the right side. It is transversely oval and measures about 0·37 × 0·26 mm. The yolk glands are very voluminous filling up the greater part of the posterior half of the body and extending along each side to the level of the middle of the pharynx. The follicles are somewhat small and are arranged in a peripheral layer, which in front of the ventral sucker extends right across the body dorsally but only for a short distance ventrally. At the level of the ovary the ventral layer begins to extend in towards the middle line overlapping the edges of the ovary and anterior testis and almost completely covering the posterior testis. Behind the latter the follicles from each side merge and completely fill the post-testicular space. There is no receptaculum seminis but the initial two or three convolutions of the uterus are packed with sperm. No Laurer's canal was observed. The uterus fills up the region between the ovary and the ventral sucker, and is confined within the space bounded by the intestinal diverticula. The eggs do not exceed 100 in number. They are brownish yellow and of moderate size, measuring 0·078–0·084 × 0·046–0·050 mm.

The systematic position of this species is a matter of some difficulty. It is obviously related to the groups of which *Lepocreadium* and *Stephanochasmus* are the chief representatives, but it presents such a combination of the characters of the two groups that it is difficult to decide to which it is more nearly related. Thus, for instance, it has the reduced ventral sucker of the Lepocreadiinae but, on the other hand, it lacks the vesicula seminalis externa and the receptaculum seminis of this group. Again it has the uterine receptaculum seminis and the large pharynx characteristic of the Stephanochasminae but it lacks the crown of cephalic spines. For the present it must be regarded as an intermediate type. It does not appear to be closely allied to any other known form. Its generic characters may be summarized as follows:

Lepidauchen n. g.

Body broad and flat; covered with spines anteriorly. Oral sucker much larger than ventral, which lies in front of the middle of body. Prepharynx short, pharynx very large, oesophagus absent. Genital aperture median, near intestinal bifurcation. Cirrus pouch short and stout. Receptaculum seminis absent. Otherwise as in Allocreadiinae

Type species: *L. stenostoma* from intestine of *Labrus berggylta.*

Hemipera ovocaudata n. g., n. sp.

(Figs. 4, 5.)

This peculiar and interesting form was found in the stomach of four out of 24 specimens of *Lepadogaster gouanii.* In all but one case only a single immature example was obtained; in the fourth three examples occurred, two of which contained eggs.

The species is small and delicate, elongated and sub-cylindrical in shape, with somewhat pointed ends. The largest specimen measured 1·54 mm. in length with a breadth of 0·56 mm. across the ventral sucker. The surface of the body is smooth and has no cuticular spines.

Both suckers are globular. The oral sucker is subterminal and measures 0·22 mm. in diameter. The ventral sucker is much larger, measuring 0·40 mm., and is situated at a distance of 0·87 mm. from the anterior end of the body, *i.e.* distinctly behind the middle.

Contiguous with the oral sucker is a medium sized pharynx having a diameter of 0·066 mm. It is followed by a very short oesophagus. The diverticula pass out at right angles to the oesophagus but after a short distance bend abruptly backwards and run down the sides of the body to the posterior end, their ends being very close together. The excretory vesicle resembles that of *Derogenes* and the Hemiuridae. The median stem, which is very narrow, divides into two near the posterior border of the ventral sucker. The paired limbs pass out towards the sides of the body but at the level of the pharynx they turn in and unite dorsal to the pharynx.

The testes are symmetrically situated near the posterior end of the body, from which they are separated by a space equal to half their length. Each slightly overlaps the corresponding intestinal diverticulum and they are separated from each other by a narrow space through which runs the excretory vesicle. They are elongated oval bodies, measuring 0·22 × 0·14 mm. Their long axes are slightly oblique.

The genital aperture lies in the middle line immediately behind

the intestinal bifurcation. The somewhat elongated cirrus pouch contains only the pars prostatica and the ductus ejaculatorius. The vesicula seminalis lies entirely outside the pouch, with which it is connected by a fairly long duct, which is bent up alongside the pouch. The pouch is divided into two parts by a distinct constriction about its anterior third. The posterior part is entirely filled with the pars prostatica. The vesicula seminalis, which lies alongside the cirrus pouch and somewhat behind it, is a little smaller than the posterior portion of the pouch.

The ovary lies to the right of the middle line immediately behind the ventral sucker. It is a small globular body of 0·09 mm. diameter. The yolk glands lie on either side of it and a little in advance. Both overlap the intestinal diverticula. Each consists of a compact ovoid mass of follicles, which is somewhat larger than the ovary but smaller than the testes (0·15 × 0·08 mm.). The yolk ducts pass behind the ovary and a fairly conspicuous yolk reservoir is formed. There is a large shell gland and a small receptaculum seminis but Laurer's canal is apparently absent. It is somewhat doubtful if the receptaculum seminis is a constant structure. In the living specimen it appeared at times to be quite distinct but sometimes it seemed to be merely a dilatation of the oviduct. In no case did it contain more than a few spermatozoa. In the preserved specimens its presence could not be detected. The uterus is of no great extent and the largest specimen contained less than thirty eggs. Possibly none were completely mature. The eggs were scattered around the ovary and along the left side of the ventral sucker. They are remarkable in possessing a single long filament extending from the anopercular pole. The filaments were directed backwards and to some extent intertwined. The eggs are slightly curved and measure about 0·10 × 0·027 mm. and the filaments about 0·2 mm.

The systematic position of this form will be discussed after the next species has been described.

Derogenoides ovacutus n. g., n. sp.

(Fig. 6.)

This is a form which bears a much greater resemblance to *Derogenes varicus* than the preceding species does, though it presents one or two marked features of difference. Numerous specimens were met with on one occasion in the stomach of *Trachinus draco*. The only other specimen of this fish which I have had an opportunity of examining was obtained from the North Sea and was not infected with this

parasite. Along with this new species there occurred a small number of specimens of *Derogenes varicus*.

It is a rather small form, mature specimens measuring only 0·6–0·9 mm. in length. 0·6 mm. appears to represent its minimum adult length as all the specimens below that were immature. Its shape is elongated, sub-cylindrical with rounded ends. The greatest breadth in an average specimen (0·73 mm. long) is 0·18 mm. The breadth, however, is fairly uniform. The cuticle is smooth and unarmed.

The oral sucker is subterminal with a small fleshy lobe projecting in front of it. It is globular with a diameter of 0·066 mm. The ventral sucker is also globular with a diameter of 0·123 mm. It lies 0·34 mm. from the anterior end, *i.e.* a very little in front of the middle of the body.

Contiguous with the oral sucker is a moderately large pharynx, measuring 0·039 mm. in diameter. The oesophagus is short and in no case longer than the pharynx. The intestinal diverticula are simple and fairly straight, reaching almost to the posterior end of the body.

The excretory vesicle resembles that in *Derogenes*, the unpaired median stem dividing some distance behind the ventral sucker (between the testes) and the paired limbs uniting dorsal to the pharynx.

The disposition of the genital glands also resembles that in *Derogenes*, the testes being approximately symmetrical with a slight tendency to obliquity. They are separated from the ventral sucker by a space of about 0·04 mm. and measure 0·12 × 0·096 mm. Their axes are oblique, the anterior pole in each case being directed outwards. Immediately behind the testes lies the large transversely oval ovary, the dimensions of which are 0·07 × 0·12 mm. Behind this again lie the globular yolk glands, which are practically symmetrical. In front of the ovary and between the posterior ends of the testes lies a small receptaculum seminis. The shell gland complex is situated on the dorsal side of the ovary.

The uterus is not very voluminous and contains only about 40 eggs. These are situated for the most part behind the yolk glands. The terminal part of the uterus passes up between the testes, over the right side of the ventral sucker and unites with the proximal end of the cirrus pouch. The eggs differ from those of *Derogenes* in having the anopercular pole drawn out into a sharp point. In their passage through the uterus the pointed end is always directed backwards. They measure 0·033–0·042 × 0·015–0·019 mm., the average being 0·038 × 0·018 mm.

The terminal part of the male organs bears a general resemblance to that of *Derogenes* but differs from it in detail. It is in the first place not so elongated, the vesicula seminalis being small and globular. The

pars prostatica is considerably shorter and the prostatic cells much fewer in number. The pseudo-cirrus pouch, however, is distinctly longer, being somewhat cylindrical instead of globular. The posterior end is slightly inflated. The genital aperture lies immediately over the intestinal bifurcation.

The two foregoing species present not a few features of considerable taxonomic interest. They obviously belong to the small group of forms, inhabiting the stomach, of which *Derogenes varicus* is the best known example. *Hemipera* differs from all the forms which may be included in this group by reason of the structure of its cirrus pouch, which contains not only the ductus ejaculatorius but also the pars prostatica. In all other members the latter is free. It presents the further peculiarity of having the testes situated behind the ovary. In this respect it resembles *Liocerca*, to which it is probably more closely allied than to any other genus.

Derogenoides, on the other hand, is a typical member of the group with a free pars prostatica and the testes in front of the ovary. It bears, indeed, a very close resemblance to *Derogenes*. The somewhat different structure of the terminal male organs, however, together with the more anterior position of the ventral sucker and the genital glands and the characteristically shaped eggs, appear sufficient grounds for excluding it from this genus.

The systematic position of *Derogenes* has for long been a difficulty. It was included in the family Hemiuridae by Lühe (1901) and its somewhat isolated position in this family was recognised by Odhner (1904) who suggested that a separate sub-family would probably be required for its reception. On the other hand, Looss (1907) definitely excluded it from this family. There can be little doubt, however, of its Hemiurid affinities. Its whole structure, apart from the absence of an appendix, gives evidence of this and there appears no very strong reason why it should for the present be excluded from this family. At the same time it displays considerable affinity with the Syncoeliinae and the inclusion of *Derogenes* within the Hemiuridae would necessitate the inclusion of this sub-family as well. Odhner (1911), indeed, has advocated the advisability of this step, including *Derogenes* actually within the sub-family Syncoeliinae. He extends the family moreover to include the Accacoeliinae and the group of which *Hirudinella clavata* is the chief representative. In this reconstructed family Looss's Hemiuridae takes the position of a sub-family.

In view of the great variety of structure which occurs in these forms Odhner's arguments in favour of a wider conception of the family

group appear to be well founded. It is particularly in regard to the terminal part of the male organs that the finer distinctions have arisen, but it seems inadvisable to allow the consideration of these to outweigh the value of other structural features. The various modifications of the cirrus pouch which are met with in the true Hemiurids appear again in the *Derogenes* group, and if the same narrow limits of classification were adopted in this group the necessity would arise of creating a number of small sub-families and of erecting the Syncoeliinae into a separate family. I am on that account in agreement with Odhner in considering that, for the present at any rate, such a scheme of sub-division would not be advantageous.

Obviously the two genera, *Hemipera* and *Derogenoides*, must be included in the sub-family Syncoeliinae, *Derogenoides* in close relation to *Derogenes* and *Hemipera* to *Liocerca*. In the event of further sub-division eventually becoming necessary it is apparent that these two pairs would form the nuclei of smaller groups, both differing from the true Syncoeliid type in having the ends of the intestines free, and the *Liocerca-Hemipera* group being further distinguished by the inverted position of the ovary and testes.

The inclusion of these two forms within the family Hemiuridae, *sensu lat.*, involves slight modifications of Odhner's amended definition, namely: Pars prostatica usually free but not in *Hemipera*. Ova 0·015–0·100 mm., usually oval, but sharply pointed at one end in *Derogenoides*, and filamented in *Hemipera*.

REFERENCES.

Looss, A. (1907). Beiträge zur Systematik der Distomen. Zur Kenntnis der Familie Hemiuridae. *Zool. Jahrbüch. Abt. f. Syst.* XXVI, 63–180.

Lühe, M. (1901). Ueber Hemiuriden. *Zool. Anz.* XXIV, 473–488.

Odhner, T. (1904). Die Trematoden des arktischen Gebietes. *Fauna arctica*, IV (2), 291–372.

—— (1911). Zum natürlichen System der digenen Trematoden. IV. *Zool. Anz.* XXXVIII, 513–531.

EXPLANATION OF PLATE XI.

Fig. 1. *Podocotyle syngnathi.* Ventral view. ×20.
Fig. 2. *Podocotyle syngnathi.* Cirrus pouch and vagina. ×80.
Fig. 3. *Lepidauchen stenostoma.* Ventral view. ×30.
Fig. 4. *Hemipera ovocaudata.* Ventral view. ×66.
Fig. 5. *Hemipera ovocaudata.* Shell gland complex. ×130. Semi-diagrammatic.
Fig. 6. *Derogenoides ovacutus.* Ventral view. ×150.


1
2
3
4
5
6

TRYPANOSOMES FOUND IN A COW IN ENGLAND.

By ALFRED C. COLES, M.D., D.Sc., F.R.S. Ed., M.R.C.P. Lond.

(With Plate XII.)

History. Trypanosomes have been cultured from the blood of cattle in many parts of the world; in Germany, Denmark, Greece, Holland, France, Russia, Japan, the Philippines, Siberia, Algeria, Tunis, the United States and Brazil. But in only a few cases have the actual parasites been found in the circulating blood. From the scanty literature at my disposal, but especially from the *Sleeping Sickness Bulletin*, I find that the following observers have found and described the trypanosomes in the blood.

S. Stockman (1910) inoculated ten pedigree English cattle going to South Africa with *Piroplasma*. Nine of them showed piroplasms in their red corpuscles. In the blood films of six of these trypanosomes were found, in one instance nine days after the inoculation, and noted for a period of eight days.

The length of the parasite was about 50 μ, the breadth 2·5 μ. The posterior extremity was pointed, and at the anterior end there was a long flagellum. The undulating membrane was very distinct. Stockman states that the parasite is not to be distinguished morphologically from *Trypanosoma theileri*. Attempts at cultivation on artificial media failed. He points out that the discovery suggests that some of the British blood-sucking flies may be capable of acting as true carriers of trypanosomes.

Frank, in July 1908, whilst examining some pathological material from an ox sent from Westerwald and suspected to contain *B. anthracis*, found actively motile trypanosomes. These were characterised especially by the drawn out and pointed posterior end resembling a flagellum.

M. Mayer (1910) points out that this belongs to the *T. theileri* group which vary in length from 30–70 μ.

Knuth and Rauchbaar (1910) examined 69 smears taken from 97 animals, 48 of which were cattle, to see if they could find *T. franki*, but with negative results.

Peter (1910), whilst Veterinary Surgeon to Liebig's Extract of Meat Co. in Uruguay, from 1904–1909, found trypanosomes in cattle. These had the following characters. Length 30–60 μ. The aflagellar end is sharply pointed, in the larger forms long and beak-like, in the smaller short and hook-shaped. The blepharoplast is longish or round and is sometimes near the posterior end, sometimes near the nucleus. The nucleus lies exactly in the middle of the body: it is most frequently oval in shape and placed somewhat obliquely, or it is round. The free part of the flagellum attains the length of 15 μ. Multiplication stages are seldom seen in the circulating blood. Most of the cattle were Herefords or Shorthorns. The natural infection was found in seven cattle and in each case the disease was detected in the slaughter-house, the presence of a splenic tumour leading to the examination of the blood and spleen pulp. In two instances piroplasmata were found as well as trypanosomes: the latter were always very scarce. Peter (1910) would place this trypanosome in the *T. theileri* group.

Paul Behn (1910), during researches on the trypanosomes occurring in German cattle, in which Knuth, Rauchbaar and Morgenstern showed the presence of trypanosomes by cultural methods, found a large trypanosome in a cow. Its total length was 55 μ, breadth 12 μ, free flagellum 12 μ. The macronucleus was placed transversely, with the blepharoplast situated 4 μ behind it. Behn was unable to find another individual in this preparation in spite of long examination.

Wrublewski (1908) described a trypanosome found in the blood of a dead Lithuanian bison.

Harold Crawley (1912) under the name of "*Trypanosoma americanum*, a common Blood parasite of American cattle," describes trypanosomes which he obtained by cultural methods from cattle in the United States. An attempt was made to discover the trypanosome in freshly drawn blood which was centrifuged, and preparations were obtained in which the leucocytes were as abundant as the red corpuscles. Six different animals were used, and a large number of fresh preparations were examined, but no trypanosomes were ever found: this nevertheless is believed to be the most efficient method. When, however, stained smears were examined trypanosomes were found in two slides out of 25. Crawley gives the following description of the trypanosomes. "As to the morphology of the blood forms, a selection of 14 gave an average

Fig. 1. Trypanosome of cow. (×1000.)

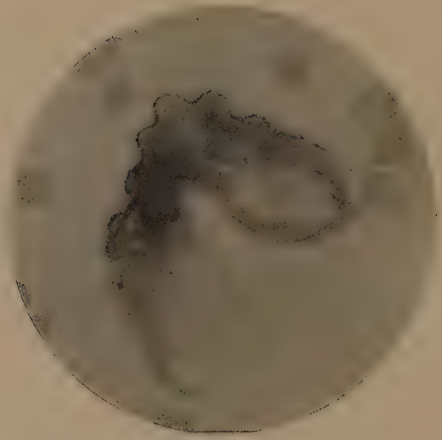


Fig. 2. Distorted stumpy form. (×1000.)

measurement of 16·8 × 3·8 μ. The trypanosomes in the circulating blood probably have a length of at least 20 μ, excluding the flagellum, and this was the size of the trypanosome that was found in the first day culture."

Principal characteristics of *Trypanosoma americanum* (from the *Sleeping Sickness Bulletin*, Vol. IV, p. 150): "It is a large trypanosome: a total length of 75 μ is by no means uncommon. The undulating membrane is very short: the kinetonucleus may be in front, alongside or behind the trophonucleus, but the two are always close together. The trophonucleus is at the junction of the anterior and middle thirds: hence the shortness of the undulating membrane."

As to the rarity of the trypanosomes, the author writes—"Here it is seen that the smallest quantity of blood to give a positive result was five drops, or 0·3375 c.c., and that this result was obtained only once out of three trials. Assuming 6,000,000 red cells and 10,000 leucocytes per cubic millimetre, we find as a possible proportion one trypanosome for 2,022,000,000 red cells and 3,370,000 whites. Hence to find the trypanosome in the circulating blood would be merely a piece of good fortune. Further, culture 444, containing 9 c.c. of blood, was negative, yet this amount of blood contains 90,000,000 leucocytes[1]."

Trypanosomes found in a Cow. For some considerable time I have been making a systematic examination of the blood of domestic animals, small mammals, birds, fish, etc., and I asked my friend Mr J. S. Wood, Veterinary Surgeon, of Parkstone, to send me any blood films from diseased animals and particularly from cases of redwater fever in cattle. These films were received air dried, unfixed and unstained, and were afterwards stained with Giemsa's stain. I should like here to express my thanks to Mr Wood for the trouble he has taken on my behalf.

After a large number of blood films had been examined with negative results, I received from Mr Wood four films taken at 9.30 a.m., June 3, from a cow presenting symptoms of redwater fever. These films showed the presence of *Piroplasma bovis* in comparatively small numbers, only about 2 % of the red corpuscles being infected. (I say a small percentage, as during 1906 I received blood films from Mr T. B. Goodall taken from a cow with redwater fever, in which 23½ % of the corpuscles contained *Piroplasma*.)

[1] See *Trypanoplasma* sp. Bowhill, 1909, and *T. rutherfordi* Hadwen, 1912, observed in Cows at Mount Lehman, British Columbia (Watson and Hadwen, *Parasitology*, this vol. p. 24, Feb. 1912). Ed.

The cow from which Mr Wood obtained the blood films was a Shorthorn about eight years old, from a farm in Dorset. On June 2nd and 3rd the urine was of a deep red colour, but during the latter day it became almost clear, being only slightly tinged.

In the evening of June 3rd at 6 p.m. four more films were taken. These were, as Mr Wood says, very badly and unevenly spread, as the cow was very restless.

These seven films were systematically examined and in one of them a perfect trypanosome was found. Subsequently in the same preparation another trypanosome was also found showing considerable distortion.

On June 6 Mr Wood took me over to see the cow and I made about 20 more films, but after a prolonged examination no more trypanosomes were detected.

Description of the Trypanosome. The parasite has the form of an almost perfect **S**, being thickest in the middle of the body and tapering towards both extremities.

The *Body* is stained a deep violet-blue colour with Giemsa and is filled with minute granules closely packed together in all but the extremities, which are quite free from granules for about 10 μ in the anterior end and for about 8 or 9 μ in the posterior end. The thickest part of the body is about the region of the nucleus, but it continues at the same width for about 16 μ, after which it gradually tapers towards the extremities, both of which terminate in fine points, especially the anterior or flagellar end. There is no difficulty in determining the exact point at which the anterior extremity of the body ceases and the free portion of the flagellum begins.

The somewhat abrupt termination of the granular protoplasm of the body gives an impression of the posterior extremity being merely a sheath. There are no indications of myonemes, although some of the granules are arranged somewhat longitudinally.

The *undulating membrane* is thrown into a number of folds and is stained a pale lilac colour, in which the granules are loosely scattered in marked contrast to their compact arrangement in the body.

The undulating membrane is bounded by a very distinct border, the attached part of the flagellum, whilst the free portion of the latter is short, measuring only about 10 μ. The trophonucleus is situated almostly exactly at the middle of the body. Its greatest diameter lies transversely to the long axis of the parasite. It measures 6 μ by 3·3 μ. Its shape is somewhat like the letter **B**, the anterior border being

straight, the posterior thrown into two slight curves. It stains a pale red colour with Giemsa. The margins are surrounded by granules, which are arranged somewhat loosely, giving the impression, so often seen in the trypanosomes of birds, of being surrounded by a lighter stained area. No definite internal structure can be made out.

The kinetonucleus is a very insignificant looking structure by no means easily seen. It is round, deeply stained, and lies in an area in which there are fewer granules. It is situated about 5 μ from the centre of the nucleus, and has a diameter of about 1·2 μ.

It is by no means easy to trace the attached portion of the flagellum directly to the kinetonucleus, although there are areas of fewer granules along its path.

The distorted trypanosome differs considerably in its general appearance from that described above. As will be seen from the figure, it is a large stumpy form, but during the preparation of the film its posterior extremity has been crushed and therefore it is impossible to give any accurate description. In this individual the flagellum does not seem to run up to the kinetonucleus but stops a short distance in front of it, the interval being made up by an exceedingly fine line which can be recognised in the photograph.

The dimensions of the perfect trypanosome are as follows: total length, 98 μ; length of body, 88 μ; free flagellum, 10 μ; distance from posterior extremity to kinetonucleus 37 μ, and to the centre of trophonucleus, 44 μ; distance between trophonucleus and kinetonucleus, 5·5 μ; diameter of body at trophonucleus, 6 μ, together with the undulating membrane, 9·5 μ.

In conclusion I should like to express my thanks to Mr J. W. Ogilvy and Mr Wm. Harding for their kindness in taking the microphotographs.

REFERENCES.

Behn, P. (1910). Ueber Entwicklungsformen des *Trypanosoma franki*. *Berliner tierärztliche Wochenschrift*, xxvi, 809–810.

Crawley, H. (1912). The Protozoan Parasites of Domesticated Animals. *Bureau of Animal Industry, U.S. Department of Agriculture.* Report No. 27 (for the year 1910), pp. 184–187.

Frank, G. (1909). Ueber den Befund von Trypanosomen bei einem in Stein-Wingert (Westerwald, Regierungsbezirk Wiesbaden) verendeten Rinde. *Zeitschr. f. Infektionskrankh. paras. Krankh. u. Hyg. d. Haustiere*, v, 313–315.

Knuth, P. and Rauchbaar, G. (1910). Zum Vorkommen von Trypanosomen bei Rindern in Deutschland. *Berl. tierärztl. Wochenschr.* xxvi, 609.

Mayer, M. (1909). Ueber *Trypanosoma theileri* und diesem verwandte Rindertrypanosomen. *Zeitschr. f. Infektionskrankh. paras. Krankh. u. Hyg. d. Haustiere*, VI, 46–51.

Peter, O. (1910). Morphologische und experimentelle Studien über ein neues, bei Rindern in Uruguay (Süd-Amerika) gefundenes *Trypanosoma*. *Beiheft z. Arch. f. Schiffs- u. Tropenhygiene.*

Stockman, S. (1910). Preliminary Note on a Trypanosome of British Cattle. *Journ. Comp. Path. and Therapeutics*, XXIII, 189–192.

Wrublewski, K. J. (1908). Ein *Trypanosoma* des Wisent von Bielowesch. *Centralbl. f. Bakteriologie*, Orig. XLVIII, 162–163.

W. Smith.

IN MEMORIAM.

WILHELM DÖNITZ.

BORN 27 JUNE, 1838, IN BERLIN.
DIED 12 MARCH, 1912, IN BERLIN.

(With Portrait, Plate XIII.)

FRIEDRICH KARL WILHELM DÖNITZ, the son of Christoph and Caroline Dönitz, was born on the 27th June, 1838, in Berlin.

As a boy he showed marked taste for natural history and ability as a draughtsman. He pursued his medical studies in 1859–64, and became assistant to Reichert at the Anatomical Institute, Berlin. During this period he occupied himself with investigations relating to histology, comparative anatomy, general zoology and biology, and teratology. His results were of such a character that in 1873 the title of Professor was conferred upon him by his government and in the same year he was called to fill the Chair of Anatomy in the newly-established Academy of Medicine in Tokio. Before proceeding to Japan he married Fräulein Martha Schirmeister.

Anatomical dissection had not until then been practised in Japan, consequently with the arrival of Dönitz a new era was opened for medical science in that country, plenty of material being available in view of the frequent executions which took place by hanging. He next occupied himself with sanitary administration especially in combatting typhoid fever and cholera and securing good water supplies. Moreover, he had much to do with the administration of various hospitals and was engaged actively in practice both as a physician and surgeon. In 1877 he went to Nagasaki owing to the outbreak of civil war. Here thousands of wounded were sheltered in the large temples and were afterwards sent by sea and under his care to Tokio. During a stay of thirteen years in Japan he travelled about and resided in different parts of

the country, his many practical duties never preventing him from following his bent for natural history. He collected an extensive herbarium, many Coleoptera, Lepidoptera and Spiders. His ability as an artist is well shown in his beautifully illustrated publications on spiders and ticks.

In 1880 he journeyed to Europe and returned to Southern Japan, where he resided at Saga.

In 1886 Dönitz returned to Berlin where he became associated with Robert Koch, first at the Hygienic Institute and afterwards at the Institut für Infektionskrankheiten, which was founded in 1891. In 1893 he directed the Bacteriological Laboratory at Bonn and concerned himself with cholera investigations. In 1896–9 he was a member of the Institut für Serumforschung und Therapie at Steglitz of which Paul Ehrlich was director. This Institute gave rise subsequently to the Institut für Serumtherapie at Frankfurt. Dönitz, however, remained in Berlin where he was put in charge of the Krankenabteilung of the Institute for Infectious Diseases, and he received the title of Geheimer-Medizinalrat. In later years he was appointed director of the Scientific Department of the Institute to which he remained attached until his death. During the many years of Robert Koch's absence in the Tropics, Dönitz acted as director in his place.

A perusal of the appended bibliography of his published works, which I believe to be complete, will convince the reader that Dönitz was a man of very wide knowledge and singular ability. It is a pleasure to acknowledge the kind aid I have obtained from the Dönitz family in writing this notice and completing the bibliography. In the latter the publications are grouped under various headings to facilitate reference.

His contributions to parasitology were of considerable importance. He handled a great part of the rich material collected by Robert Koch in many parts of the world, more especially the collections of mosquitoes and ticks. In 1901–3 he described 12 new species of *Anopheles*. His papers on ticks (1905–1910) constitute contributions of the first order, for he threw a great deal of light upon the subject of their classification, apart from giving short and accurate descriptions of new or known species. He was the first to point out that the ornamentation in *Amblyomma* and other Ixodidae depended upon their internal structure and could, in consequence, be relied upon as a means of classification. With his wide knowledge of zoology he also possessed to a conspicuous degree the ability to seize upon the essential characters

possessed by a species, and those of us who have occupied ourselves with this group will always be grateful for the great help he gave us in dealing with a difficult problem.

His papers dealing with bacteriology and immunity relate to cholera, tuberculosis, tetanus, diphtheria, typhoid and leprosy, to antitoxins and their standardization.

Considering that for long periods of time Dönitz was alternately a busy practitioner, a stimulating teacher and an effective administrator, it fills one with astonishment to view the great number and range of his scientific contributions. Personally, he was most unassuming, amiable, and ever ready to help those needing his advice. The writer remains deeply indebted to him for his active interest and ready help when working upon ticks both in Berlin and Cambridge. I possess numerous letters from Dönitz written to within a few days of his death—all of these letters contained valuable suggestions and help.

Owing to failing strength and to recuperate prior to undergoing an operation which had become inevitable, he, shortly before his death, went to Bordighera. Returning to Berlin after an absence of five weeks, he addressed his last letter to me: "Gestern bin ich frei von Bronchitis, von der Riviera zurückgekommen und konnte mich wieder meines Lebens erfreuen, wenn nicht die unangenehme Operation in Aussicht stände." The letter was full of detailed information about specimens regarding which I had consulted him, and he seized the earliest and last opportunity of sending his help where it was needed. Within less than a week he had passed away, purulent peritonitis following upon the operation he underwent for malignant disease of the intestine—of the existence of which he appears to have happily been oblivious.

Dönitz leaves a widow, a daughter and a son[1] to mourn his loss. The interment took place at the Friedhof des Invalidenhauses.

That Dönitz made numerous friends goes without saying, and witness is borne thereto in the able obituary notice by Geheimrat Gaffky which appeared in the *Deutsche medicinische Wochenschrift*. The death of Dönitz removes a distinguished man of science from our midst, and it is with a keen sense of personal loss that the writer offers this tribute to his memory.

G. H. F. NUTTALL.

[1] Dr Alfred Dönitz, Privatdocent in Surgery, Berlin.

LIST OF PUBLICATIONS BY WILHELM DÖNITZ.

(1864—1910.)

GENERAL ZOOLOGY.

INCLUDING ANTHROPOLOGY, ANATOMY, HISTOLOGY AND TERATOLOGY.

Dönitz, W. (1864). *De tunicae intestinorum villosae epithelio.* 32 pp. Small 8°. M.D. Thesis, 15. I. 1864. Berlin: G. Lange.

—— (1864). Ueber die Schleimhaut des Darmkanals. *Archiv f. Anat., Phys. u. wiss. Med.* Heft 3–4, pp. 367–406, pl. x.

—— (1864). Mariotte'scher Fleck bei markhaltigen Nervenfasern der Retina. *Ibid.* pp. 741–743, pl. xvi B.

—— (1865). *Beschreibung und Erläuterung von Doppelmissgeburten.* 38 pp. 3 pl. 8°. Berlin: C. Unger. Repr. from *Arch. f. Anat., Physiol. u. wissensch. Med.* Leipzig.

—— (1865–66). Beschreibung und Erläuterung von Doppelmissgeburten. *Archiv f. Anat., Physiol. u. wiss. Med.* pp. 113–126, 129–151, pls. ii and iii; Part 2, pp. 512–520, 610–628, pls. xii and xiii; (1866) Part 3, pp. 518–544, pls. xiii and xiv.

—— (1866). Ueber den typischen Bau der Echinodermen. *Ibid.* pp. 406–413, pl. xi B.

—— (1866). Ueber die Darmzotten. *Ibid.* pp. 757–762.

—— (1866). [Zeigte das aus Helgoland stammende Hautskelet eines Seeigels (*Echinus sphaera* O. F. Müller) vor, welches in eigentümlicher Weise missgestaltet ist.] *Sitzungsber. d. Ges. naturf. Freunde,* 17 April. pp. 12–13.

—— (1867). Ueber die Gelenke an der Rücken- und Afterflosse der *Teuthies* C. val. *Arch. f. Anat., Phys. u. wiss. Med.* pp. 210–220, pl. vii A.

—— (1867). Ueber die sogenannten amöboiden Bewegungen und die Cohnheim-schen Entzündungserscheinungen. *Ibid.* pp. 394–396.

—— (1867). [Zeigte drei Exemplare von *Antennarius urophthalmus* Bleeker vor.] *Sitzungsber. d. Ges. naturf. Freunde,* 19 Februar.

—— (1867). [Berichtete über seine Untersuchungen der *Noctiluca miliaris.*] *Ibid.* 19 November, pp. 29–30.

—— (1868). Ueber *Noctiluca miliaris* Sur. *Arch. f. Anat., Phys. u. wiss. Med.* pp. 137–149, pl. iv.

—— (1868). Ueber *Noctiluca miliaris.* Erwiderung an Herrn Prof. V. Carus. Berlin [22 Dezember].

—— (1868). [Short communications]: Spricht unter Vorzeigung von Präparaten und Zeichnungen über einige niedere, für die Fauna von Helgoland neue Seetiere. *Sitzungsber. d. Ges. naturf. Freunde,* 21 April; spricht über sogenannte amöboide Bewegungen und die Cohnheim'schen Entzündungserscheinungen. *Ibid.* 16 Juni, pp. 17–18; sprach über die Eckzähne der Lemuriden. *Ibid.* 15 Dezember, p. 32.

—— (1869). [Short communications etc.]: bemerkte im Anschluss hieran [*Gurlt* über einen Parasiten], dass Geschwülste in der Kreuzbeingegend entweder aus

krankhaften Neubildungen bestehen, oder aber ein zweites Individuum darstellen etc. *Sitzungsber. d. Ges. naturf. Freunde*, 16 Februar ; sprach über den feineren Bau der Muskelfasern wirbelloser Tiere. *Ibid.* 20 Juli, pp. 26–27 ; sprach unter Vorzeigung von Präparaten über das von Nitzsch entdeckte *Siphonium*. *Ibid.* 19 Oktober, p. 33 ; zeigte einen Schakalschädel mit einem überzähligen Lückenzahn vor. *Ibid.* 21 Dezember, p. 41.

Dönitz, W. (1869). Ueber das Remak'sche Sinnesblatt. *Arch. f. Anat., Phys. u. wiss. Med.* pp. 600—622, pl. xv.

—— (1870). Ueber die sogenannte Chorda der Ascidienlarven und die vermeintliche Verwandtschaft von Wirbellosen und Wirbeltieren. *Archiv f. Anat., Phys. u. wiss. Med.* pp. 761–764 ; also [sprach über die vermeintliche Stammverwandtschaft zwischen Ascidien und Wirbeltieren etc.]. *Sitzungsber. d. Ges. naturf. Freunde*, 19 Juli, pp. 47–51.

—— (1870). [Sprach über bisher unbekannte, frühe Entwicklungsstadien von Radiolarien und über die Biologie der Alveolen derselben.] *Sitzungsber. d. Ges. naturf. Freunde*, 19 November, pp. 71–72.

—— (1871). Beobachtungen über Radiolarien. *Archiv f. Anat. und Phys.* pp. 71–82, pl. ii.

—— (1871). Ueber *Cordylophora lacustris*. *Ibid.* pp. 430–433, pl. xi B.

—— (1871). Ueber eigentümliche Organe an den Magenstücken der Siphonophoren. *Ibid.* pp. 83–89, pl. iii.

—— (1871). Beiträge zur Kenntnis der quergestreiften Muskelfasern. *Ibid.* pp. 434–446, pl. xii.

—— (1871). [Short communications] : Sprach über bewegliche Organe, die er bei Schwimmpolypen gefunden hat. *Sitzungsber. d. Ges. naturf. Freunde*, 21 Februar ; bemerkt dass das Anwachsen von vollständig abgelösten Teilen auf einem fremden oder auf demselben Mutterboden nicht mehr ein ausschliessliches Vorrecht der Botanik sei. Behandelt Transplantation an Hautstückchen auf Wundflächen. *Ibid.* 21 Februar, pp. 18–19 ; sprach über den Bau der *Nieren des afrikanischen Elephanten*. *Ibid.* 21 März, pp. 25–26 ; bemerkte im Anschluss an Herrn Brauns Vortrag, dass ihm Herr General Ramsay (früher Resident der englischen Regierung in Nepal) die Mitteilung gemacht habe, dass in Nepal ein vorzügliches Papier aus der Rinde einer Art Kellerhals [Daphne] bereitet werde. *Ibid.* 21 März, pp. 26–27 ; sprach über die Muskeln der Schwimmpolypen (Siphonophoren). *Ibid.* 20 Juni, pp. 57–59 ; sprach über einen Brachwasserpolypen, *Cordylophora lacustris*. *Ibid.* 17 Oktober, pp. 81–82.

—— (1872). [Short communications] : Legte mehrere Schädel mit auffallenden Abweichungen im Gebiss vor. *Sitzungsber. d. Ges. naturf. Freunde*, 18 Juni, p. 54 ; sprach über die *Entwicklung der Zoospermien bei Schwimmpolypen*. *Ibid.* 18 Juni, pp. 54–55 ; sprach über die geographische Verbreitung der Zibethyäne, *Proteles lalandii*, etc. *Ibid.* 16 Juli, p. 63.

—— (1872). Ueber die Nieren des afrikanischen Elephanten. *Archiv f. Anat. u. Phys.* pp. 85–89, pl. ii B.

—— (1873). Ueber die Halswirbelsäule der Vögel aus der Gattung *Plotus*. *Ibid.* pp. 357–360, pl. ix A.

—— (1876–1877). [Short communications] : Uebersendung einer Anzahl von Separatabdrücken über die Abstammung der Japaner. *Archiv d. Berl.*

Anthropol. Ges. p. 10. Sitzung am 15 Jan. 1876; Ueber den Vogelfang in Japan. Vortrag, gehalten am 7 April, 1877. *Kleinere Mitteilungen*, pp. 2–3; Ueber drei verschiedene Typen unter Japanerschädeln. Vortrag gehalten am 30 Dez. 1877. *Ibid.* pp. 1-2.

DÖNITZ, W. (1887). [Spricht, unter Vorzeigung zahlreicher archäologischer Gegenstände, über vorgeschichtliche Gräber.] *Verh. d. Berliner Anthropol. Ges.* Sitzung am 22 Jan. pp. 114–126.

—— (1909). Jahresbericht für 1909. *Ges. d. naturf. Freunde*, 11 Okt. pp. 583–587.

ENTOMOLOGY AND ARACHNOLOGY.

(SEE ALSO UNDER PARASITOLOGY.)

—— (1887). Ein singender Schmetterling. *Berlin. Ent. Zeitschr.* XXXI, 9–10.

—— (1887). [Short communications]: neue und auffalende Beispiele von Anpassung und Nachahmung bei Arthropoden, bez. bei Schmetterlingen und Spinnen. *Sitzungsber. d. Ges. naturf. Freunde*, 21 Juni, pp. 97–102; in Japan gemachten Beobachtungen über Copulation von Spinnen. *Ibid.* 19 April, pp. 49–51; Lebensweise zweier Vogelspinnen aus Japan. *Ibid.* 18 Jan. pp. 8–10.

—— (1889). [Short note]: Feinde der Schmetterlinge mit Rücksicht auf den Schutz, welche letzteren durch Anpassung und Nachahmung gewährt wird. *Ibid.* 19 Nov. pp. 185–186.

—— (1890). [Short communications]: Tabellarische Uebersicht über den Nachtköderfang während eines Sommers bei Berlin, etc. *Sitzungsber. d. Berlin. Ent. Ges.* 17 Feb. p. 2; einen Fremdkörper am Kopfe eines Schmetterlings. *Sitzungsber. d. Ges. naturf. Freunde*, 18 März; eine Serie aus Japan...eine *Macroglossa*. *Sitzungsber. d. Berlin. Ent. Ges.* 28 April, p. vii; zeigt eine *Acherontia atropos*, resp. zwei Höhlenkäfer. *Ibid.* 8 Dez. p. 15; zeigt *Stegnascophyct tetraquetrana* Hw. *Sitzg. d. Berlin. Ent. Ver.* 17 März, p. iv, *Ibid.* 31 März, p. vi; über Aufweichen von Schmetterlinge. *Ibid.* 24 Feb. p. iii.

—— (1891). [Short communications]: Erkennungszeichen der beiden Geschlechter bei *Papilio*. *Sitzungsber. d. Berlin. Ent. Ver.* 9 März, p. 22; zeigt einen Zwitter von *Smerithus populi*. *Ibid.* 16 März, p. 22; [demonstrates] eine grosse Anzahl Tafeln mit von ihm selbst in Japan gezeichneten Spinnen. *Ibid.* 13 April, p. 24; [regarding] *Erastria*. *Ibid.* 4 Mai, p. 24; [regarding] *Satunia pyri*. *Ibid.* p. 27.

—— (1892). [Short communications and demonstrations]: Legt eine grössere Anzahl von ihm selbst präparirten Raupen vor. *Ibid.* 11 Januar, pp. 2–3; Erfahrungen mit der Zucht von *Cidaria miata* (Linn.) und *C. siterata* Hfn., *Ibid.* 18 Januar, p. 13; zeigt eine früh ausgekommene *Selenia bilunaria* u.s.w. *Ibid.* p. 4; zeigt eine Reihe präparierter Schmetterlingsflügel zum Studium des Geäders. *Ibid.* 21 April, p. 7, über *Argynnis leodice*. *Ibid.* 19 Mai, p. 8; zeigt eine bei Königs-Wusterhausen gefangene *Gnophia quadra* ♀. *Ibid.* 15 Sept.; hat beobachtet, das in Japan die Apfelsinenbäume von *Agrilus p. arg.* geschädigt wurden. *Ibid.* 6 Okt. p. 10; zeigt eine Anzahl in der Jungfernheide gefangener *Cidaria testaceata*. *Ibid.* 20 Okt. p. 11; hat eine zweite Generation der *Cidaria*

unangulata gezogen. *Ibid.* 20 Okt. pp. 11–12; *Parnassius delphius* Ev. ab. *Caecus*. *Entom. Nachrichten*, XVIII, 193–195.

Dönitz, W. (1893). [Short communications and demonstrations]: zeigte in Alkohol aufbewahrte Larven der Mantiden *Hypsicorypha juliae* und *Blepharis meudica* *Sitzungsber. d. Berlin. Ent. Ver.* 22 Feb. p. 8; zeigt eine kleine Deltoide aus Japan, *Zanclognatha trilinealis* Brem. *Ibid.* p. 9; zeigt von ihm in Japan erbeutete Varietäten von *Lasiocampa pini*. *Ibid.* 13 April, p. 2; zeigt Lepidopteren an Turkestan. *Ibid.* 20 April, p. 2.

—— (1895). [Short communications]: "Adolf Streckfus." *Berlin. Ent. Ver.* XI. 373–374; über Lycaenidenraupen. *Sitzungsber. d. Berlin. Ent. Ver.* 10 Jan. p. 2; über *Breplos*. *Ibid.* 25 April, p. 10; hält einen längeren Vortrag über die Feststellung der Art in der Entomologie. *Ibid.* 2 Mai, pp. 11–14.

—— (1896). [Short communications]: referirt über die Erfahrungen von Duclaux über die künstliche Beschleunigung des Ausschlüpfens der Seidenraupeneier. *Sitzungsber. d. Berlin. Ent. Ver.* 21 Jan. p. 1; seine Erfahrungen über die Aufbewahrung von Insekten in Formol. *Ibid.* 9 Juni, p. 2; Bemerkungen über Dasselfliegen. *Ibid.* 23 April, pp. 7–8; über die Wirkung der Gifthaare an Insekten. *Ibid.* 30 April, p. 8; Discussion über *Belphos notum*. *Ibid.* 21 Mai, p. 9; der Litteratur über *Ocneria dispar* betreffend. *Ibid.* 1 Okt. p. 16; [discussion and report] vom ostasiatischen Spinner *Dionychopus niveus*. *Ibid.* 1 Okt. pp. 15–16; Reports on Bruce's investigations on *Glossina* in S. Africa. *Sitzungsber. d. Berlin. Ent. Ges.* 21 Mai, p. 10.

—— (1897). [Short communications]: Bildung der Copulationsorgane eines der wichtigsten Mittel um hybride Befruchtung zu vermeiden (in Bezug auf *Urania* Species oder Lokalform). *Sitzungsber. d. Berlin. Ent. Ver.* 7 Jan. pp. 1–2; Lebensweise der Raupe von *Boletobia fuliginaria*. *Ibid.* 30 Sept. p. 16.

—— (1898). [Address]: Ueber die californische sogenannte San José-Schildlaus. *Sitzungsber. d. Berlin. Ent. Ver.* p. 4.

—— (1899). Ueber die Echtheit der Farbentöne der braunen und grünen Form der *Ornithoptera zalmoxis*. *Insektenbörse*, XVI. 22.

PARASITOLOGY.

MOSQUITOES AND MALARIA, INSECTS AND DISEASE, TICKS. SEE ALSO UNDER ENTOMOLOGY.

—— (1901). Ueber Stechmücken. *Insektenbörse*, XVII; Vortrag über die Beziehungen der Stechmücken zur Malaria (Berlin. Ent. Ver.). *Ibid.* XVIII. 34–36.

—— (1902). Beiträge zur Kenntniss der *Anopheles*. *Zeitschr. f. Hyg.* XLI. 15–88, pls i, ii.

—— (1903). Beiträge zur Kenntniss der *Anopheles*. II. Mitteilung. *Zeitschr. f. Hyg.* XLIII. 215–238.

—— (1905). Ueber eine neue afrikanische Fliege mit parasitischen in der Haut von Ratten lebenden Larven, *Cordylobia murium*. *Sitzungsber. d. Ges. naturf. Freunde*, No. 10, pp. 245–254, pl.

—— (1905). Die Zecken des Rindes als Krankheitsüberträger. *Sitzungsber. d. Gesellsch. naturf. Freunde*, No. 4, 105–134, 1 pl.

Dönitz, W. (1906). Zecken als Krankheitsüberträger. Vortrag gehalten am 25 Nov. 1905. *Bericht der Senkenbergischen Naturf. Ges. in Frankfurt a. M.* pp. 39–52.

—— (1906). Ueber Afrikanische Zecken. *Sitzungsber. d. Gesellsch. naturf. Freunde*, No. 5, 143–148, 8 figs.

—— (1906). Die Haematozoenkrankheiten. *Naturw. Wochenschr.* Jena, xxi, 177–182.

—— (1907). *Die wirtschaftlich wichtigen Zecken mit besonderer Berücksichtigung Afrikas.* 8°. 127 pp. 38 figs and 6 pls. Leipzig: J. A. Barth.

—— (1907). Die Texasfieberzecke, *Boophilus annulatus*, und das Ixodinengenus *Margaropus*. *Sitzungsber. d. Gesellsch. naturf. Freunde*, No. 6, pp. 187–192.

—— (1907). Insekten als Verbreiter von Krankheiten: Die Zecken. *Ber. ü. d. XIV. Internat. Congr. f. Hygiene u. Demographie*, ii. 179–188.

—— (1908). Feinde der Landwirtschaft und der Menschheit. *Die Woche*, No. 9, pp. 373–374.

—— (1909). Ueber das Zeckengenus *Amblyomma*. *Sitzungsber. d. Gesellsch. naturf. Freunde*, No. 8, 440–482, figs 1–15.

—— (1910). "Die Zecken Südafrikas." In Schultze's *Zool. u. Anthropol. Ergebnisse einer Forschungsreise im westl. u. zentr. Südafrika ausgeführt in den Jahren* 1903–1905. Jena: Gustav Fischer, 399–494, pls. xv. (farbig), xvi A, xvi B, und xvii.

—— (1910). Zwei neue afrikanische Rhipicephalusarten. *Sitzungsber. d. Gesellsch. naturf. Freunde*, No. 6, 275–280, figs 1–4.

MEDICINE AND SURGERY, PATHOLOGY, BACTERIOLOGY AND IMMUNITY.

—— (1879). Ueber Bereitung und Anwendung von Eisenalbuminat. *Berlin. klin. Wochenschr.* xvi, 535.

—— (1886). Bemerkungen zur Cholerafrage. *Zeitschr. f. Hyg.* i. 405–420.

—— (1888). Zur Technik der Operation des Brustkrebses. *Berlin. klin. Wochenschr.* xxv, 544.

—— and Lassar, O. (1889). Ueber Mycosis fungoides (*Granuloma fungoida*). *Virchow's Arch.* cxvi, 301–309, 1 pl.

—— (1891). Ueber die Wirkung des Tuberkulins auf die experimentelle Augentuberkulose des Kaninchens. *Deutsche med. Wochenschr.* xvii, 1289–1291.

—— (1894). Ueber die Arbeiten der bakteriologischen Untersuchungstation in Bonn in Jahre 1893. *Centralbl. f. allgem. Gesundheitspfl.* xiii, Heft 1 und 2, pp. 37–44.

—— (1895). Ueber das Verhalten der Choleravibrionen im Hühnerei. *Zeitschr. f. Hyg.* xx, 31–45; also in *Riv. internaz. d' igiene*, Naples, vi, 435.

—— (1897). Ueber das Antitoxin des Tetanus. *Deutsche med. Wochenschr.* xxiii, 428–430.

—— (1898). Untersuchungen über die Werthbestimmung des gewöhnlichen Tuberkulins. *Klinisches Jahrb.* viii.

—— (1898). Nachträgliche Bemerkungen zu den Untersuchungen über die Werthbestimmung des gewöhnlichen Tuberkulins. *Klin. Jahrb.* viii, Heft 2.

DÖNITZ, W. (1898). Versuche mit experimentell diphtherisch gemachten Meerschweinchen durch Diphtherieheilserum. *IX. Internat. Congr. of Hygiene and Demography*, Madrid.

—— (1899). Ueber die Grenzen der Wirksamkeit des Diphtherieheilserums. *Arch. internat. de Pharmacodynamie*, v, 425–435.

—— (1900). Bericht über die Thätigkeit des Kgl. Instituts für Serumforschung und Serumprüfung zu Steglitz, Juni 1896 bis Sept. 1899. *Klin. Jahrb.* VII, 359.

—— (1900). Welche Aussichten haben wir, Infektionskrankheiten, insbesondere die Tuberkulose, auszurotten? *Berlin. klin. Wochenschr.* XXXVII, 364–367, 388–392. Also *Med. Chir. Centralbl.* Wien, XXXV, 253–257, 269–270. (Address delivered 22. III. 1900 at the Charité.)

—— (1900). Behandlung der Lepra. *Berlin. klin. Wochenschr.* XXXVII, 793–794.

—— (1901). Besichtigung des Kgl. Instituts für Infektionskrankheiten, Berlin N. Nordufer-Föhrerstrasse. *Brandenburgia*, Novemberheft pp. 292–302.

—— (1901). "Die Infektion" in *Die deutsche Klinik am Eingange des 20. Jahrhunderts in akademischen Vorlesungen*, herausgegeben von v. Leyden und F. Klemperer, Part I, Lecture 3, pp. 35–63. Vienna: Urban und Schwarzenberg.

—— (1903). Ueber die im Institute für Infektionskrankheiten erfolgte Ansteckung mit Pest. *Berlin. klin. Wochenschr.* XL, 609–612. (Discussion), 621, 644, 67. Also abstract in *Deutsche med. Wochenschr.* XXIX, Ver.-Beil. 227–243.

—— (1903). Die Immunität. *Deutsche Klinik*, Berlin u. Wien, I, 553–586.

—— (1904). Die Behandlung der Lungentuberkulose. *Zeitschr. f. ärztl. Fortbildung*, Jena, I, 377–382.

—— (1904). Wertbemessung der Schutz- und Heilsera. In *Handb. d. Pathogenen Mikroorganismen*, herausg. von Kolle u. Wassermann. Jena, pp. 570–591.

—— (1904). Bericht über die Thätigkeit des Instituts für Infektionskrankheiten zu Berlin während des Jahres 1903. *Klin. Jahrb.* Jena, XIII, 351–366.

—— (1904). "Ueber die Quellen der Ansteckung mit Typhus, nach Berliner Beobachtungen," in *Festschrift zum 60. Geburtstag von Robert Koch.* Jena: Gustav Fischer, pp. 297–314.

—— (1910). Die Spandauer Cholerafälle. *Med. Klinik*, VI, 1469–1470.

—— (1910). Robert Koch's Lebenswerk. *Naturw. Wochenschr.* N.F. IX, 417–422.

THE HERTER LECTURES.

I. *SPIROCHAETOSIS.*

LECTURE DELIVERED ON THE HERTER FOUNDATION, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MARYLAND, U.S.A., 8 OCTOBER, 1912.

By GEORGE H. F. NUTTALL, M.A., M.D., Ph.D., Sc.D., F.R.S.
Fellow of Magdalene College; Quick Professor of Biology, Cambridge.

Under the term "Spirochaetosis" are included those diseases of man and animals due to the spiral microorganisms known as spirochaetes. I shall confine myself to those which produce blood infection—the relapsing fevers—in which a remarkable periodic increase and decrease in the number of the spirochaetes is observable corresponding to alternating rises and falls of the host's body-temperature. Authority is divided as to whether the spirochaetes are Protozoa or Bacteria, and the matter is a fruitful theme of discussion upon which I shall not enter here. Judged, however, from their pathological effects and their prompt reactions to immune sera and certain drugs, they show a pronounced affinity to Protozoa and exhibit phenomena not hitherto observed in Bacteria.

As in trypanosomiasis, spirochaetosis is readily induced by inoculation with infected blood, and may thus be communicated almost indefinitely from animal to animal. In one series, for instance, I transmitted *S. duttoni*, with apparently undiminished virulence, through 100 mice. Again, as in trypanosomiasis (*T. lewisi*, *T. evansi*), infection may take place by feeding.

Transmission of Spirochaetes by Arthropods.

Investigations conducted during the last few years have demonstrated conclusively that the blood-inhabiting spirochaetes are, in a number of instances, transmitted by blood-sucking Arthropods, and I propose to deal chiefly with these results since they are of great practical importance to preventive medicine.

Spirochaetosis in Birds.

In the year 1891, Sacharoff, in the Transcaucasus, demonstrated that a spirochaete, called by him *Spirochaeta anserina*, was the cause of a very fatal epidemic disease in geese. The spirochaetes appeared in the birds' blood shortly before the onset of symptoms, multiplied enormously, and disappeared at the approach of death. He transmitted the disease to geese and fowls by inoculation. In the year 1903, Marchoux and Salimbeni, working in Brazil, observed a similar disease in fowls, and since that date fowl spirochaetosis has been recorded from many parts of the world, the causative agent being now generally known as *Spirochaeta gallinarum*. We know to-day that spirochaetosis in fowls occurs in S. E. Europe, in Asia, Africa, S. America and Australia, and, in all places where the disease exists is found, what Marchoux and Salimbeni were the first to show to be the carrier, the tick, *Argas persicus*. I have seen blood-films and determined the tick from many different places where the disease has been recorded. Personally, there is no longer any doubt in my mind as to the identity of *S. anserina* Sacharoff and *S. gallinarum*. *A. persicus* has accompanied the fowl in its distribution in many parts of the world, but the fowl has got rid of the pest in colder climates as the tick is unable to develop at low temperatures.

Spirochaetosis in fowls is a very fatal febrile disease; the mortality in a yard may attain 40–100 %. The disease begins with diarrhoea, followed by loss of appetite and somnolence. The birds' feathers appear ruffled, the comb pale, the birds cease to perch, and, as the disease advances, they lie prostrate upon the ground. Death may occur suddenly during a convulsive attack. The disease occurs at times in a chronic form, the emaciated birds developing paralytic symptoms after apparent recovery. Death takes place in anywhere from 3 to 15 or more days, according to the type of the disease, the body-temperature at the time of death being frequently subnormal. Whereas, in chronic cases, the liver and spleen appear atrophied, these organs are much enlarged in acute cases, the liver showing fatty degeneration and at times focal necroses. The fowl spirochaete from Brazil kills geese in five to six days after inoculation, and produces a fatal infection in ducks, guinea-fowls, turtle-doves, and other birds.

Thanks to the kindness of Dr Marchoux, I was able, at an early date, to confirm his and Salimbeni's results with infected *A. persicus* (= *miniatus*) which he sent me from Brazil. Since that date Marchoux, Borrel, and others, also Hindle, in my laboratory, have materially

advanced our knowledge of the mechanism whereby the tick infects the fowl. Without wearying you with the details of each experimenter's work, I may summarize it as follows:

The ticks are best rendered infective if they are maintained at a temperature of 30–35° C. after they have fed upon blood containing the spirochaete. If kept at a low temperature, 15–18° C., the spirochaetes disappear very soon from the ticks alimentary tracts, and they may bite birds repeatedly without infecting them. They may, however, be rendered infective after three months if placed at 30–35° C.: the spirochaetes then reappear in their coelomic cavity, as may be shown by cutting off one of the tick's legs and examining the coelomic fluid which exudes from it upon a slide.

When the spirochaetes first enter the tick they soon disappear from the gut, a certain number degenerate, whilst others traverse the gut wall and enter the coelomic cavity to circulate all over the body. A number of them die in this situation as evidenced by the frequent presence in the coelomic fluid of pale, scarcely visible, non-motile spirochaetes which are difficult to stain. The spirochaetes next enter the various organs, especially the cells of the malpighian tubules and sexual organs, in which they break up into a large number of small particles or coccoid bodies which multiply by fission and give rise to large agglomerations which can be seen very distinctly in stained specimens (Heidenhain stain). The coccoid bodies may also be found within the lumen of the gut and malpighian tubules and in the excreta. In the act of feeding, the tick occasionally voids excrement and exudes a few drops of secretion from coxal glands situated in the first inter-coxal space, the fluid pouring out of a wide duct and being rapidly secreted from the freshly imbibed blood serum. This fluid, as well as the salivary and intestinal secretion of *Argas*, contains an anti-coagulin, as I showed with Strickland. The coxal fluid dilutes the escaped excrement and facilitates its getting into the wound inflicted by the tick. This is doubtless the usual mode of infection, the coccoid bodies in the excrement gaining access to the blood of the host and afterwards developing into spirochaetes, though the latter development has not actually been followed. Marchoux and Couvy (1912) state that infection may, however, take place without coxal secretion being voided. The bird begins to show symptoms after a period of incubation of about four days following upon the bite of the infected tick.

Although it was denied that the spirochaete of the fowl is transmitted hereditarily to the offspring of *A. persicus*, I expressed the opinion

some years ago[1] that there was every probability that it would be found to be transmitted hereditarily as in *Ornithodorus moubata*. Hindle has recently confirmed this supposition. Coccoid bodies are found within the malpighian cells of the embryonic tick, as described by Leishman for *S. duttoni* in *O. moubata*. If the eggs are maintained at 37° C., the coccoid bodies grow out and assume a form which suggests that they are on the way to forming spirochaetes. The spirochaete stage occurs in the coelomic fluid of the tick, but not within its body cells. I may add here that *A. reflexus* has been shown by Shellack (1908) to transmit the fowl spirochaete.

Human Relapsing Fever in Tropical Africa.

Although David Livingstone (1857) was the first to report upon pathogenic effects following upon the bite of the tick we know to-day as *Ornithodorus moubata*, it was not until the year 1905 that Dutton and Todd, in the Congo, and shortly afterwards, Robert Koch, in German East Africa, demonstrated that this tick transmitted spirochaetosis to man. The British authors made the important observation that the *S. duttoni* is transmitted hereditarily to the offspring of the tick, a fact confirmed by Koch, who discovered that 5–15 %, and at times 50 %, of the ticks harboured the parasite. Koch captured the ticks at resting places along caravan routes and in places outside the regular routes. Apparently, owing to German East Africa having been opened to trade for a much longer period than the Congo, the tick appears to be much more widely distributed in East Africa than in the Congo. Dutton and Todd state that in the Congo it only occurs along routes of travel. I have examined a large number of specimens of this tick from various parts of Africa, and would note that its geographical distribution is far wider than our present records show for the distribution of relapsing fever in man. There is every reason to fear, therefore, that an extension of the disease will follow with time, unless the natives learn even better than they do to shun the "tampan." In fact, I have an interesting observation to note in this connection which bears out my contention. It emanates from the Rev. John Roscoe, of Cambridge, who gave me the information last year. This gentleman was a missionary in Uganda, where he lived for many years at Kampala in a native-built house having reed walls supported by the usual wooden pillars. To quote his words: "Some of the pillars were in rooms, not in the walls,

[1] Harben Lectures, 1908.

and it was at the bases of two of these pillars in the room used as a dining room that I noticed the ticks in the year 1896 or about that time. For several years I continued to live in the same house and suffered no harm from them. In more recent years, that is, about 1903 or 1904, both Europeans and natives have suffered from 'Tick Fever' (Spirillum) in houses which were built on either side of the site on which my old house stood. It has been affirmed that the ticks in these houses are the cause of the fever; I can only conclude that in previous years they were innocuous and that they have become nocuous since 1896." I do not know of any similar observation having as yet been recorded.

The disease has repeatedly been transmitted to experimental animals, rats, mice and monkeys, by means of infected ticks, and in a number of cases unwittingly to experimenters in European laboratories. I may, in this connection, instance the case of Mr Merriman, in my laboratory, who suffered from the disease in consequence of being bitten by two *O. moubata* (first-stage nymphs) whose biology he was studying. He did not know he had been bitten by the ticks until after two days when he showed me two characteristic bites upon his forearm. His attack followed 16 days after the bites were inflicted, the incubation period being four to six days longer than is usual.

Of 25 monkeys with which Möllers experimented in Berlin no less than 20 died of spirochaetosis. There is, therefore, no possible doubt about the tick being the carrier of the disease.

Möllers' observations were of fundamental importance in relation to the etiology of the disease. He proved that ticks continue to harbour the parasite even after repeated feeds upon clean animals: thus ten out of 12 monkeys were infected in succession by one lot of ticks which were fed upon them. His stock of ticks had died down to a low point toward the end of the series or the positive results would doubtless have continued longer. A tick may remain infective a year-and-a-half or longer after its initial infective meal of blood. He proved, moreover, that the parasites in the tick are transmitted hereditarily to the third generation when the ticks were fed throughout upon clean animals. Another observation possessing considerable interest is that of Manteufel (1910) that the ticks apparently acquire immunity to spirochaetal infection. Hindle has since found that about 30 % of the *moubata* sent to me from Uganda failed to become infected. It is conceivable that the stage of the disease or of the spirochaete's development at which the tick imbibes the parasites may have some influence upon the number

of ticks which become infected, as noted by Miss Muriel Robertson for *Trypanosoma gambiense* in *Glossina palpalis*, to which reference will be made in the next lecture. Such a condition might well account for some of the immunity which is stated to occur. We know that there are marked variations in the viability of spirochaetes in relapsing fever blood preserved *in vitro*. Thus, Novy and Knapp (1906) found that *S. recurrentis* (American strain) survived for 30 to 40 days in defibrinated blood drawn from a rat during the onset of the disease, whereas they only survived 24 hours in blood drawn during the decline.

Although Dutton and Todd, Balfour, and others observed the breaking up of spirochaetes into minute granules in the body of ticks, Leishman was the first to follow the process more clearly. He proved that the coxal secretion was anti-coagulant and non-infective, and that the excreta were infective by inoculating them into animals. He found that only when *moubata* voided excreta in the act of biting that animals under experiment became infected. He therefore concluded that the mode of infection is contaminative through the tick's excreta and not active through its proboscis. Experiments which I carried out, and which were extended and reported upon by Hindle in my laboratory, completely confirm the results of Leishman. If the internal organs of an infected *moubata* are carefully dissected out and well washed in sterile salt solution, it is found that the gut, together with its contents, the malpighian tubes, the sexual organs and excrement are infective when emulsified and injected into a susceptible animal. The coxal secretion always, and the salivary glands in most cases, give negative results. The few positive results with salivary gland inoculations may well be referred to experimental error, in that the glands, in the process of dissection, may easily become contaminated by spirochaetes derived from other organs and be imperfectly cleansed in the process of washing. Inoculations with emulsified eggs of *moubata* have also given positive results as might be expected, for spirochaetes have been found in them by a number of authors; Koch (1905) and Carter (1907) being among the first to demonstrate their presence in this situation.

After being ingested by the tick, the spirochaetes usually disappear from the lumen of the gut in about nine to ten days, but they reappear if the tick is placed at 35° C. They are then found in the coelomic fluid and their subsequent behaviour is similar to that described in the fowl spirochaete.

It is highly probable that other species of *Ornithodorus* play a like part in the etiology of relapsing fever in other parts of the world than

those in which *moubata* occurs, the latter being a purely African species. *O. savignyi*, which is indistinguishable from *moubata* at a casual glance, and which also occurs in Africa, at Aden and in India, has been found by Brumpt to convey a spirochaete derived from cases of human relapsing fever occurring in Abyssinia. *O. turicata* is suspected in connection with relapsing fever in Colombia, and *O. talaje*, I have no doubt, might play a similar part in Mexico and Central America whence I have received specimens. Lately, both Leishman and myself have received specimens of *O. tholozani* from Quetta, India, where it was suspected of being a vector, but experiments carried out with the few living examples which reached Leishman have proved negative. Again, from the fact that *A. persicus*, as tested experimentally by Sergent and Foley (1908), in the Sud-Oranais, Africa, serves as a host for spirochaetes of human origin, we may conclude that this species, which frequently attacks man, may also communicate relapsing fever under suitable conditions. Sergent and Foley found the spirochaetes present in the coelomic fluid of this tick for two days, after which they disappeared.

That neither the tick nor the spirochaete is specifically adapted to the other is a matter of considerable importance which has been revealed by recent research. In view of the morphological similarity of the supposedly different species of spirochaetes and their individual variations in virulence, we may well doubt if any of the "species" are valid. As I pointed out four years ago, the various specific names given to the spirochaetes causing relapsing fever in man may be used merely for convenience *to distinguish strains or races* of different origin[1]. They cannot be regarded as valid names, in the sense of scientific nomenclature, for virulence and immunity reactions are not adequate tests of specificity. Under experimental conditions *O. moubata* has served for the transmission not only of *S. duttoni* and two other so-called species, *S. recurrentis* and *S. novyi*, which affect man in the Old and New World respectively, but it has also been found to transmit the fowl spirochaete. *S. duttoni*, moreover, has been successfully transmitted to rats by *Haematopinus spinulosus*, the common rat-louse. There is every reason to suppose that a spirochaete capable of adapting itself either to a tropical African tick or to a rat-louse occurring all over the world, will be able to accommodate itself to a variety of vertebrate hosts; and we know in fact, from laboratory tests, that a considerable number of animals

[1] *S. recurrentis* may be the only true species; the name *recurrentis* has priority over *S. obermeieri*. Other so-called species are *duttoni*, *rossi* or *kochi*, *novyi*, *berbera*, *carteri*, etc.

are susceptible to infection with *S. duttoni*, various species of monkeys, rats, mice, rabbits, guinea-pigs, sheep, goats, horses and dogs, etc. having been successfully infected.

Transmission of Relapsing Fever by Pediculus and Cimex.

It has long been supposed that vermin are responsible for the transmission of relapsing fever in Europe. Flügge (1891) appears to have been the first scientific writer to suggest this possibility, and Tictin (1897) supposed that bugs (*Cimex lectularius*) might transmit the disease by their bites or by being crushed and their contents entering the skin through excoriations due to scratching. He infected monkeys with the contents of bugs removed 24 hours after they had fed on relapsing fever blood. Karlinski (1902) and likewise Schaudinn observed the survival of spirochaetes in bugs for 30 days or more. Christy (1902) and Breinl, Kinghorn and Todd (1906) failed to transmit spirochaetosis by bugs. In experiments of my own (1907) it was found that *S. duttoni* survived six days in the bug at 12° C., but only for six hours at 20–24° C. Similar results were obtained by *S. recurrentis* (from Russia). The parasites appeared to be merely digested by the bug, the rate of digestion being governed by the temperature at which the insects were maintained. In but one experiment did I succeed in transmitting relapsing fever to a mouse by means of bugs. In this case, I used 35 of the insects, and transferred them directly from an infected to an uninfected mouse, interrupting their feed upon the first animal and allowing them to complete it upon a second clean mouse. We may, therefore, conclude that bugs can occasionally transmit relapsing fever.

We have, on the other hand, conclusive proof that lice are concerned in the transmission of the disease. The first important evidence in this connection dates from Mackie (1907), in India. This author records an outbreak of relapsing fever amongst school children, in which 137 out of 170 boys and 35 out of 114 girls, were attacked. The boys were found to be more infested with vermin than were the girls. An examination of the lice removed from the boys showed 24 % of them to contain spirochaetes, whereas only 3 % of the lice collected from the girls contained these microorganisms. As the epidemic increased among the girls their verminous condition became more evident, as the epidemic decreased among the boys the lice were found less frequently upon them. Mackie noted that the spirochaetes multiplied within the gut of the lice and that they could be found in the ovary, testis and

malpighian tubules of the insects. He concluded that infection might result from the insects regurgitating the contents of their alimentary canal into the wound in the act of feeding.

Sergent and Foley (1908) next observed the presence of *Pediculus vestimenti* upon the persons of nearly all patients affected with relapsing fever in Sud-Oranais, N. Africa, and they observed spirochaetes in the bodies of the lice. Subsequently (1910), they found these lice associated with every case they observed in Algeria.

The most convincing observations are, however, those published in a short paper this year by Nicolle, Blaizot and Conseil (1912). They note, in respect to its epidemiology, that relapsing fever affords a striking similarity to typhus fever. The disease extends in a similar manner, it occurs in the same places, when it enters hospitals it does not spread, sparing the nurses and physicians who have to deal with the patients who have been cleansed, whereas it attacks those who have to handle the patients at their entry into the hospital. In both diseases, as observed in Tunisia, lice are invariably found on the patients.

Nicolle and his colleagues obtained negative results when they attempted to transmit the disease through the *bites* of infected lice placed upon experimental monkeys and five persons (two of whom were the authors), although both men and monkeys were exposed to thousands of bites collectively. Upon studying the behaviour of the spirochaetes in the lice (*P. vestimenti* and *P. capitis*), they found that they disappear and afterwards reappear. But few can be detected in the gut five to six hours after the infective feed, and none are discoverable microscopically when 24 hours have elapsed. After about eight to 12 days, however, actively motile spirochaetes reappear in the louse; at first they are short, but later they resemble those seen in the blood. Such spirochaetes are observable in lice up to the 11th day, and possibly longer. Monkeys inoculated with the contents of lice, crushed on the 15th day after the infective feed, developed relapsing fever.

We know that all persons infested with lice are addicted to scratching themselves, whereby they excoriate their skin and frequently crush the lice upon their bodies. In this manner their hands and finger-nails become infected with the body contents of the lice including the spirochaetes, and these gain a ready entrance through the excoriated skin, thereby infecting the individual. One of the authors, having excoriated his skin, smeared the contents of an infected louse upon the lesion, and succeeded thereby in infecting himself, the disease developing after a period of incubation lasting five days. In one experiment,

infection followed the placing of the contents of a louse upon the conjunctiva in man. In nature, it might well happen that the soiled hand might travel to the eye and produce infection in a similar manner. The authors proved, moreover, that the spirochaetes are transmitted hereditarily to the offspring of the infected lice, for they found that eggs, laid 12 to 20 days after the infection of the parent lice, contained the spirochaete. The larvae issuing from these eggs likewise contained spirochaetes. By incubating the eggs at 28° C., the larvae hatched out on about the 7th day. When the eggs or larvae were crushed and inoculated into a monkey the latter became infected.

We still lack detailed information regarding the behaviour of the spirochaetes in the lice and their offspring; possibly it is similar to that recorded for *S. duttoni* in *O. moubata*. The main point may, however, be now regarded as established that lice (both *P. vestimenti* and *P. capitis*) transmit relapsing fever and are presumably the ordinary vectors in most parts of the world. These discoveries are naturally of the greatest practical importance, in view of the prevention of relapsing fever.

I shall here digress to say a few words about the biology of lice infesting man, since you will find no precise information about it in the literature, except for the observations made by my Demonstrator, Mr Cecil Warburton, in Cambridge. The latter has made the only accurate observations hitherto recorded for *P. vestimenti* in conjunction with an investigation we undertook on behalf of the Local Government Board, the results of which were published in their Reports for 1910. Mr Warburton found that *P. vestimenti* (= *corporis*) lives longer than *P. capitis* under adverse conditions. This is doubtless due to its living habitually on the clothing, whereas *capitis* lives upon the head where it has more frequent opportunities of feeding. He reared a single female upon his own person with self-sacrificing enthusiasm, keeping the louse enclosed in a cotton-plugged tube with a particle of cloth to which it could cling. The tube was kept next to his body, thus simulating the natural conditions of warmth and moisture under which these creatures thrive. The louse was fed twice daily whilst it clung to the cloth upon which it rested. The female lived one month. She copulated repeatedly with a male which died on the 17th day, and was replaced by a second male which likewise entered into copulation and survived the female. Copulation commenced five days after the female emerged and the process was repeated a number of times, sexual union lasting for hours. The female laid 124 eggs

within 25 days. The eggs hatched after eight days under favourable conditions, such as those under which the female was kept; they did not hatch in the cold. Eggs kept near the person during the day and hung in clothing by the bedside during the winter in a cold room, did not hatch until the 35th day. When the larvae emerge from the egg they feed at once if given a chance to do so. They are prone to scatter upon the person and abandon the fragment of cloth to which the adult clings. The adult stage is reached on the 11th day after three moults occurring about every fourth day. Adults enter into copulation five days after the last ecdysis. The adults reared by Warburton lived about three weeks after the final moult, and the "egg to egg" period is reckoned at about 24 days. Unfed *P. vestimenti* adults died quickly at any temperature; only one specimen survived in a feeble condition until the fifth day. Unfed larvae died in 36 hours.

To this we may add that Nicolle and his colleagues find that both *P. vestimenti* and *P. capitis* survive longest when maintained at 28° C., in a damp atmosphere, being fed twice a day.

I have allowed myself this digression, dealing with *Pediculi*, because Warburton's results are doubtless unknown to many, and these parasites have only lately crept into prominence especially with regard to the etiology of typhus fever and relapsing fever. It is of importance to note how long the eggs may survive in view of the hereditary transmission of the spirochaetes in lice. It is obvious that the disinfection of verminous clothing is indicated as a preventive measure, and that those coming in contact with patients suffering from these diseases should promptly change their clothing and inspect their persons carefully after exposure with a view to avoiding the bites of infected lice.

Spirochaetosis in Cattle.

The discovery of spirochaetosis in cattle is due to Theiler after whom the causative agent, *S. theileri*, has been named. The parasite is transmitted by the tick *Boophilus decoloratus* in Africa. Laveran and Vallée, to whom Theiler sent the infective ticks, reproduced the disease experimentally in France. That the ticks in this case become infected hereditarily goes without saying, for the infective ticks used by the French authors were larvae hatched from eggs laid by females which had fed on cattle harbouring the spirochaetes in South Africa. We lack observations to show if the ticks may remain continuously infective through several generations, as seen in *S. duttoni*-infected *O. moubata*.

About 14 days after such infective larvae are placed upon cattle, the latter develop spirochaetosis, but the infection appears to be mild. In Laveran and Vallée's experiments spirochaetes were only present in the blood for four days. Four days later, however, the animal developed piroplasmosis, proving that the ticks had transmitted a double infection. Koch, working in Africa in the same year (1905), likewise observed spirochaetosis in cattle and reported finding the spirochaetes in the eggs of a species of tick which he found upon the affected animals.

This exhausts the list of spirochaetes concerning whose mode of conveyance we have definite knowledge. There are, however, a number of different animals infected by spirochaetes which are doubtless transmitted in a similar manner. Horses occasionally harbour spirochaetes, and so do sheep in Africa, and, judging from inoculation experiments, these spirochaetes are probably identical with *S. theileri*. Bats, as Nicolle and Comte found in Northern Africa, suffer from a typical relapsing fever due to *S. vespertilionis* which may be conveyed by several of the numerous ectoparasites infesting these animals: *Argas vespertilionis* and lice would naturally suggest themselves to me as being the probable vectors.

Spirochaeta muris, occurring in rats and mice, and *S. gondi* Nicolle, 1907, occurring in a small African rodent (*Ctenodactylus gundi*) are both transmissible by blood inoculation and presumably in nature are transmitted by ectoparasitic arthropods.

Cultivation of Spirochaetes.

Another important step in our knowledge concerning spirochaetes is that they can be cultivated *in vitro*. All efforts to cultivate them under ordinary conditions, suitable for the great majority of Bacteria, have given negative results in the hands of many bacteriologists all over the world. Levaditi (1906), it is true, succeeded in cultivating *S. gallinarum* and *S. duttoni* in collodion sacks placed, according to the usual technique, in the peritoneal cavity of rabbits. Under these conditions the spirochaetes multiplied and lived for 73 days or more. Successful cultivation *in vitro* has, however, only been recently accomplished by Noguchi (August, 1912), by adding a few drops of citrated rat or mouse blood, containing the spirochaetes, to sterile ascitic or hydrocele fluid (10 to 15 c.c.) in tubes containing pieces of freshly excised rabbit's kidney. Precautions against bacterial contamination are imperative;

it is best to collect the infected blood at the 48th to the 72nd hour of the disease, and the tubes should be maintained at 36° C. He experimented with *S. duttoni* and two strains of *S. recurrentis* which he calls *kochi* and *obermeieri*. *S. duttoni* was still virulent after the 9th transplantation; *S. kochi* was transplanted 29 times, subcultures being made every four to nine days, the maximum growth being attained about the 9th day. This strain appeared to lose its virulence by prolonged culture. *S. obermeieri* attained its maximum growth on the 7th day, and was still virulent after having attained the 7th subculture.

To sum up, then, we have represented in the blood-inhabiting spirochaetes of warm-blooded animals a group of microorganisms which, under natural conditions, are mainly conveyed by blood-sucking ectoparasites within which they undergo a process of development and in which they are hereditarily transmitted. Spirochaetes are not specialized parasites. Infection may take place through the skin or mucous membrane to which the spirochaetes gain access by being deposited thereon in the arthropod's dejecta or by the infested individual scratching or rubbing himself with hands which have become contaminated with the contents of the vermin which they have crushed. The lesions produced by the bites of the arthropods and the excoriations inflicted upon the individual by himself greatly facilitate the entrance of the spirochaetes.

THE HERTER LECTURES.

II. TRYPANOSOMIASIS.

LECTURE DELIVERED ON THE HERTER FOUNDATION, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MARYLAND, U.S.A. 9 OCTOBER, 1912.

By GEORGE H. F. NUTTALL, M.A., M.D., Ph.D., Sc.D., F.R.S., *Fellow of Magdalene College; Quick Professor of Biology, Cambridge.*

The term trypanosomiasis is applied to-day to a group of diseases affecting vertebrates and caused by parasitic Protozoa belonging to the family Trypanosomidae. These parasites occur chiefly in the blood plasma and the typical form possesses an elongated body, an undulating membrane, a single nucleus, a blepharoplast, and a chromatic filament running along its length from near the blepharoplast, along the margin of the undulating membrane, to terminate freely at one end of the body. The latter has a somewhat spiral form, the protoplasm being alveolar and at times showing a granular structure. An axial filament has been described as occurring in some forms. One species, *Trypanosoma equiperdum*, occurs largely in the lymph, and another, *T. gambiense*, may invade the cerebrospinal fluid. They are actively motile organisms, multiplying usually by longitudinal or multiple division. Numerous species of trypanosomes have been successfully cultivated in vitro in the presence of haemoglobin, and by this means many trypanosomes have been discovered in animals in which these parasites occur in such scanty numbers that their presence cannot be detected microscopically. Thus, a large percentage of birds and cattle in different parts of the world have been found to harbour trypanosomes in their blood.

It would appear from the evidence gathered of late years that the majority of these parasites are conveyed from host to host by blood-sucking ectoparasites, either arthropods or leeches, whereas in some cases there is clear proof (partly experimental) that infection may

take place directly from vertebrate to vertebrate through the contact of abraded or even healthy mucous membranes and skin. Although much has been written regarding a supposed sexual phase in the life-cycle of trypanosomes, we are still in the dark as to its occurrence; the little evidence there is points, however, to its possible occurrence in the invertebrate hosts which serve as vectors, so that, at any rate provisionally, these may be regarded as the definitive hosts of the parasite. Infection may be brought about under experimental conditions by the inoculation of blood containing trypanosomes, or by applying blood to abraded or intact mucous membrane and skin. In nature, the infection of dogs by feeding on animals dead of Surra (due to *T. evansi*) has frequently been observed, and similar results have been obtained experimentally. Dourine in horses is commonly communicated in nature in the act of coitus, and Koch suspected that the trypanosome of sleeping sickness (*T. gambiense*) might be similarly communicable in man, a supposition which has since been strengthened by laboratory experience. In one instance, there is evidence that non-biting insects,—*Musca domestica*,—may serve as vectors; I refer to the equine disease "murrina" at Panama, due to *T. hippicum*, which is not apparently transmitted unless the animals show wounds of the skin upon which flies may alight and thus convey the parasites directly from host to host. In this case, also, it is possible that infection may take place by coitus.

Judging from earlier evidence which proved that different species of trypanosomes could be readily communicated from animal to animal by inoculation, in some cases by the transference of minute quantities of blood, it was generally supposed that the parasites, under natural conditions, were communicated by biting flies in a purely mechanical manner. Experimental evidence on this point appeared, moreover, to bear out this supposition, for in a number of instances trypanosomes were transmitted from diseased to healthy animals by removing a fly from an infected animal upon which it was feeding and, soon after, transferring it to a healthy animal upon which it was allowed to complete its meal. For a long time the classical experiments of Bruce, in Zululand, were accepted as evidence of the mechanical transference of *T. brucei* by *Glossina morsitans*, he having found that infected flies captured in nature did not infect healthy horses if the flies were prevented from feeding upon them for 24 hours or more after they had been captured. Although some authors, notably in India, still regard *mechanical* transference by biting flies (*Stomoxys, Tabanus, Culicidae*)

as of importance in the spread of infection, the general trend of opinion to-day is to regard such transference as possible but of slight significance in the epidemiology of trypanosomiasis.

Owing to the widespread interest evoked by recent advances in tropical medicine, a large number of observers in all parts of the world have devoted much attention to the study of microscopic parasites occurring in the blood of vertebrates. The result has been that we now know of a vast number of hosts which harbour trypanosomes in their blood, and the literature relating to new species of these haematozoa has grown to be one of considerable magnitude. The general tendency has been to consider each species of animal as the carrier of a species of trypanosome peculiar to itself, this being doubtless due in part to the supposedly specific character of the adaptation shown by the parasite to its host. For a long time, one of the best known trypanosomes, *T. lewisi*, was regarded as peculiar to rats, and it is only recently that it has been shown to be also capable of living parasitically in other rodents. We know now that certain species of trypanosomes possess a wide range of pathogenicity, such forms as *T. brucei*, *T. gambiense*, *T. evansi*, and others being pathogenic for many different species of mammals. In some cases, morphological characters sufficiently differentiate the species, but our confidence in some of these characters has been shaken since we know that a species of trypanosome may alter its appearance in changing its host. Whereas the immunity reactions have been used to differentiate species of trypanosomes, a means of distinguishing species to which no zoologist will agree, we now know that this means of differentiation cannot be relied upon since the virulence (an obscure character) of the parasites can be considerably modified experimentally.

Of the discoveries which have been made of recent years with regard to trypanosomiasis, those which concern the mode of infection by blood-sucking ectoparasites unquestionably spring into prominence both because of their great scientific interest and their great practical bearing upon preventive measures designed especially to protect men and the more valuable domesticated animals against trypanosome infections. Sleeping sickness and Nagana in Africa are amongst the most deadly diseases known, and I shall commence by outlining what we know to-day regarding the way in which they are spread.

Sleeping Sickness.

Trypanosoma gambiense, the cause of sleeping sickness in Uganda and the West Coast of Africa, is conveyed by *Glossina palpalis*. It has been estimated that 0·03 to 0·34 % of wild *palpalis* in the endemic area in Uganda are infective. Infective flies have been captured on the shores of Lake Victoria Nyanza which have been uninhabited by man for three years, and they have been found on islands from which the human population has been removed for ten months. There is no evidence that *T. gambiense* is hereditarily transmitted to the offspring of an infected fly, and it cannot be assumed that *palpalis* in nature can live for any such period. We were, therefore, forced to conclude that animals inhabiting this region must be susceptible to infection and capable of harbouring the trypanosome. It is now definitely established that certain animals do serve as *reservoirs* whence the flies may derive the parasite in the absence of man. The latest reports prove that antelope (bush-buck, reed-buck and water-buck) may harbour the trypanosome in nature. Antelope kept under observation have been seen to recover from any clinical manifestations of the disease and to appear perfectly healthy for 12 to 22 months, but they continue to harbour *T. gambiense* during this period and possibly longer, and throughout this long interval of time they may, as has been experimentally shown, infect clean *palpalis* which have been raised in the laboratory. Although there is evidence that with time the antelope's blood grows less virulent, as tested by inoculation into susceptible animals, and that antelope acquire a form of immunity, they harbour virulent *gambiense* for a sufficient length of time to maintain the parasite in nature in the absence of man. As in other diseases, so with trypanosomiasis, it is the *chronic cases* which serve as "reservoirs" for lengthy periods, and are most dangerous in relation to the spread and persistence of the disease in the region affected. The relation of game animals in respect to sleeping sickness in man is therefore similar to that of game in relation to Nagana in domesticated animals; in both cases game may serve as a reservoir whence the Glossinas draw their infection. The wide range of pathogenicity possessed by *T. gambiense* renders it certain that it must, in nature, find reservoirs in other animals than antelope. In fact, the trypanosome has been recovered from cattle, monkeys (*Cercopithecus*, twice) and dogs under natural conditions. A large series of animals has been proved to be susceptible to infection by inoculation with *T. gambiense* of human origin. Without, perhaps,

giving an exhaustive list, I would mention that, in addition to the animals already noted, the chimpanzee, macacus, lemur, cat, pig, goat, sheep, hedgehog, mouse, rat, guinea-pig, rabbit, horse and donkey have been found susceptible to infection with *T. gambiense*. On the other hand, birds, reptiles and amphibia appear to be immune. The parasite has been conveyed experimentally to susceptible animals either by flies captured in a wild state or by clean flies purposely infected in the laboratory. Monkeys, sheep, goats and the duikerbok have been successfully infected in this manner.

Experiments carried out with *G. palpalis* in captivity have shown that but a limited number—about 5 to 6 %—become infective after feeding upon blood containing *T. gambiense*. It is worthy of note, in this connection, that Ross and Milne have shown that *T. rhodesiense* exhibits periodicity in respect to numbers in the blood. Miss Muriel Robertson has just reported what appear to be negative periods in monkeys suffering from trypanosomiasis. Although trypanosomes can be found in their circulation they do not appear to have reached a stage in their development when they are capable of infecting *Glossina*. She reports, moreover, that a greater proportion (up to 21 %) of *G. palpalis* become infected if the flies are starved for several days after imbibing trypanosomatous blood. In such flies a certain number of trypanosomes always degenerate and die but others soon begin to multiply rapidly throughout the gut, and this multiplication has been seen to continue within the fly's gut up to the 95th day, being maintained by repeatedly feeding the fly upon clean blood. The trypanosomes disappear from the fly's proboscis very soon after the insect has partaken of infected blood. The parasites occur in a variety of forms within the gut, they have not been found in the coelomic cavity, and it is only after a period of 25 to 28 days following the infective meal that they appear in the salivary glands of the insect. During the few hours immediately following an infective meal, the fly may transmit the trypanosome, this being doubtless due to parasites ejected from the proboscis before it has become cleaned. Then follows a period of 25–28 days during which the fly is incapable of producing infection. It is only when the parasites appear in the salivary gland that the insect becomes infective. The parasites in these glands resemble *T. gambiense* as seen in mammalian blood and they persist in the glands as long as the fly lives. Experiments made by inoculating the contents of flies into susceptible animals at various periods after an infective meal have given concordant results with those just mentioned. When the gut contents of a fly are injected

into an animal within two days after it has been fed on trypanosome-containing blood, the animal becomes infected; from the third day onwards the results of similar inoculations are negative until about the 25th day, when injections of either the gut content or salivary gland emulsion produce infection and these organs continue to be infective as long as the fly lives in captivity, *i.e.* up to the 98th day or longer[1]. *Trypanosoma rhodesiense*, now recognised as distinct from *T. gambiense*, is more closely allied to *T. brucei* than to *T. gambiense*. It is conveyed by *G. morsitans* and occurs in N.E. Rhodesia and Nyasaland in cases of sleeping sickness in man. Apart from morphological differences, it has been found to be more virulent than *T. gambiense* and to occur more plentifully in the blood of man and animals infected with the parasite. The high degree of virulence possessed by *T. rhodesiense* suggests that it is a new variety or species. Water-buck, hartebeest, mpala, wart-hog and native dog have been found to serve as reservoirs in the sense previously described. Flies captured in a wild state or raised clean and then infected in the laboratory have been shown to transmit the trypanosome. The fly becomes infective in 11–15 days (Kinghorn and Yorke, 1912, in N. Rhodesia) to 33 days (Taute, 1911, at Tanganyika), and, coincidentally with its becoming infective, the flagellates appear in the fly's salivary glands. Only about 5 % of captive flies fed upon trypanosomatous blood become infective, but, as in the case of *T. gambiense* and *G. palpalis*, the fly, once infected, remains infective as long as it lives, and it does not "clean itself" of parasites after repeated feedings on trypanosome-free blood.

Nagana.

Trypanosoma brucei is conveyed by *G. morsitans* and *G. palpalis*. The well-known disease, Nagana, to which this trypanosome gives rise, has been repeatedly transmitted by means of *G. morsitans* captured in a wild state in Africa. The parasite possesses a wide range of pathogenicity and can be transmitted for an indefinite period from animal to animal by inoculation of blood. We still maintain a strain of *T. brucei* in Cambridge which came from Zululand 15 years ago in a dog suffering from Nagana, and it has been maintained by passage from animal to animal throughout these years apparently with undiminished virulence. The greatest sufferers from Nagana are

[1] *Glossina palpalis*, a ♀, has been observed to live 227 days in captivity. This fly lives on an average 4½ months in captivity.

apparently horses and dogs, man being unaffected. Once infective *G. morsitans* remains infective doubtless as long as it lives. Tested under experimental conditions, this fly has proved infective 88 days after first imbibing the trypanosome. Through experiment with flies bred in captivity and consequently "clean" to start with, it has been shown that *G. palpalis* may also transmit *T. brucei*. As in *T. gambiense* and *T. rhodesiense*, it has been found that this fly does not become infective until about the 18th day and remains infective up to the 66th day and no doubt considerably longer.

Some other Glossina-transmitted Trypanosome Infections.

Trypanosoma dimorphon and *T. pecaudi* also appear in the salivary glands when the flies become infective. These trypanosomes are transmitted by *G. tachinoides* and *G. longipalpis*; *T. dimorphon* may also be conveyed by *G. morsitans*.

T. cazalboui is transmitted by several species of *Glossina* (*morsitans*, *palpalis*, *longipalpis* and *tachinoides*)[1]. When laboratory-bred flies are allowed to feed upon blood containing this trypanosome, 20–70 % of them become infected. *Glossina palpalis* becomes infective after 6–7 days[2] or after 17 days[3], *morsitans* on the ninth day[2] or on the 21st to 30th day[3]. After the fly feeds the parasites assume a crithidia-like form (48 hours) and remain attached by the flagellar end to the labrum or hypopharynx, the infective forms resembling trypanosomes *remain confined to the region of the fly's proboscis*. Infection has been produced by inoculation of animals with the proboscides of infected flies. The flies presumably remain infective for life, *palpalis* having been found to convey the trypanosome to susceptible animals 75 days after having infected itself.

In all the species of trypanosomes which have been enumerated (*gambiense*, *rhodesiense*, *brucei*, *dimorphon*, *pecaudi* and *cazalboui*) we have no evidence that the parasites are transmitted to the offspring of the flies which serve as vectors. In all cases the trypanosomes may occasionally be transmitted mechanically by the fly for a brief period after it has imbibed infective blood, and this is followed by a more or less lengthy period during which the fly is uninfective. Then, coincident with the appearance of parasites resembling the blood forms of the trypanosomes in the salivary glands or proboscis (*T. cazalboui*),

[1] *T. vivax* is regarded as identical with *T. cazalboui*.

[2] According to French observers.

[3] According to British observers working in a different locality with *T. vivax*.

the flies become infective and remain infective indefinitely. There is evidence that certain of these trypanosomes favour definite species of *Glossina* as hosts; if it were not so the flagellates would be even more widely distributed geographically than they are at present.

Chagas' Disease.

Schizotrypanum cruzi Chagas, 1909, the cause of trypanosomiasis in Brazil, is transmitted by a reduviid bug, *Conorhinus megistus*. This vector occurs all over Brazil in badly kept clay and wooden houses and is a night feeder, the adult insect being able to fly. The bug moults five times before attaining sexual maturity in about 324 days. The bug becomes infective eight daysafter feeding upon infected blood and remains infective over a year. A female may live over 57 days without food.

The parasites, which are numerous in the blood in acute cases, multiply in the midgut of the bug. They are at first rounded, then crithidia-like, they then assume the trypanosome form in which they occur in the gut and salivary glands of the bug. Infection occurs through the infected saliva of the bug introduced in the act of biting, but the bug's excreta are also infective when fresh. I may add that Brumpt has recently observed the development of *T. cruzi* in *Cimex lectularius* and *C. boueti*.

The disease is communicable to dogs, cats, rabbits, guinea-pigs, rats, mice, and monkeys (*Cercopithecus ruber*, *Hapale* and the Sajou). It is peculiar compared to other forms of trypanosomiasis, the thyroid gland being at times much enlarged (goitre-like). In some chronic cases there occur motor and cardiac disturbances, convulsions, infantilism and idiocy, etc.

Trypanosoma boylei Lafont, 1912.

Another interesting parasite of a reduviid bug, *Conorhinus rubrofasciatus*, has been reported upon this year by Lafont. The insect, which attacks man in Mauritius and Réunion, has been found to harbour flagellates, the intestinal tract of 50 to 80 % of the bugs containing the parasites. Lafont infected rats and mice by intra-peritoneal injection with the gut contents of the bugs. In rats the parasites remain confined to the peritoneal cavity whence they disappear in about 30 hours. In mice, on the other hand, the flagellates appear in the blood stream in from five to seven hours after inoculation and persist there for one to five days, after which they

disappear and the mice usually die. When mice harbouring the trypanosomes in their blood were bitten by the bugs, the flagellates resumed the forms (leptomonas, crithidia and trypanosome) which they originally possessed prior to entering the body of the mouse.

The rat trypanosome (T. lewisi) and its mode of transmission.

Whereas in the *Glossina*-transmitted trypanosomes the parasites enter fresh hosts through the flies' proboscides, we have another method of infection in the case of the rat trypanosome. *Trypanosoma lewisi* is conveyed by several species of flea and by the rat louse; it is world-wide in its distribution and occurs in 25–100 % of *Mus decumanus* captured in a wild state. Rats may harbour the parasite in sufficient numbers in their blood to render them demonstrable microscopically for a period lasting from a week to seven months. The usual vectors are unquestionably the common rat-fleas (*Ceratophyllus fasciatus* and *Ctenophthalmus agyrtes*) although the parasite is transmissible by other species of flea (*Ctenocephalus canis* and *Ctenopsylla musculi*) as has been demonstrated experimentally. Rat-lice (*Haematopinus spinulosus*) may occasionally serve as vectors.

Trypanosoma lewisi, after being imbibed by the flea, multiplies rapidly, chiefly in the hindgut and rectum of the insect. The parasites assume a crithidia-like appearance and occur in large bunches attached by their flagellar ends to the epithelium, or they occur in cyst-like masses within degenerating epithelial cells. Subsequently, the parasites resemble the blood forms as seen in the rat. They may then be found in vast numbers crowding the hindgut and rectum of the flea. The latter becomes infective in four to seven days and it may remain infective for 45 days or longer. The flea does not infect the rat through its proboscis, and the parasites are not found in the flea's salivary glands. Infection can take place in three ways: the flea harbouring the infective forms of the flagellates may be (*a*) crushed and devoured by the rat, (*b*) the rat may lick its fur upon which an infected flea has just dejected, (*c*) the rat may lick and infect with flea dejecta the wound produced by the insect. Fleas in the act of feeding frequently eject excreta which may be loaded with the flagellates in the infective stage. We have conclusive experimental evidence to prove that infection may occur in these different ways. We have then, in the flea, an entirely different mode of trypanosome transmission as compared to what we have seen in *Glossina*.

Leech-transmitted Trypanosomes of Fish, Reptilia and Amphibia.

Trypanosoma granulosum, occurring in the eel, developes in a leech, *Hemiclepsis marginata.* The flagellates at first multiply actively in the leech's stomach and afterwards in the intestine, where crithidial forms occur. Finally, the flagellates reassume the trypanosome form and appear in the proboscis-sheath of the leech. The latter only becomes infective when the flagellates appear in this situation.

The Trypanosomes of the gold-fish, bream, perch and rudd all develope in *Hemiclepsis marginata.* They multiply enormously in the crop, undergoing a great change in appearance, being tadpole-like in form and having a crithidia-like arrangement of the nuclei. After the 8th day, slender trypanosomes appear, and after the 10th day they gather progressively in the leech's proboscis-sheath, where they cease to divide. The leech's bite is now infective and the flagellates are cleaned out of the sheath during the process of biting. The time when the leech becomes infective depends entirely upon the rate of the leech's digestive processes, it may be delayed to the 35th day, or longer. The leech still continues to harbour the trypanosomes; others present in the gut succeed those that disappear from the proboscis after each feed.

This leech may produce a mixed infection in fish for it also transmits *Trypanoplasma cyprini* which occurs in gold-fish and tench. This parasite divides rapidly in the crop, slender forms appearing on the 2nd day and advancing on the 6th day so as to accumulate in the leech's proboscis-sheath in vast numbers. The flagellates attach themselves in this situation by their flagella and tend to crowd forward as the leech's digestion approaches completion, with the result that the leech may completely clean itself of parasites at a single feed. It is worthy of note that the trypanoplasm does not materially alter its morphology in the leech.

Trypanoplasms have also been transmitted by the leech, *Piscicola geometra,* and a number of trypanosomes occurring in fresh and salt water fish (*T. danielewskyi, T. soleae, T. rajae, T. cotti*) have been transmitted experimentally by different species of leeches (*Hemiclepsis, Pontobdella,* etc.).

Trypanosoma inopinatum, which occurs in the green frog, is similarly conveyed by a leech. In this case, according to Brumpt, the flagellates are transmitted hereditarily to young leeches, thus offering a marked exception to what takes place in all other trypanosomes whose vectors have been determined. The leech concerned is *Helobdella algira,* and

by means of this vector the trypanosomes have been successfully conveyed to *Rana esculenta* in which the flagellate does not occur in nature.

Finally, I would mention *T. vittatae* which is parasitic in a tortoise (*Emyda vittatae*) in Ceylon. This flagellate is transmitted by a leech (*Glossosiphonia*) in which it appears to behave in a similar manner to the fish trypanosomes above enumerated.

The general trend of recent work has gone to prove that, in the majority of instances, the trypanosomes of vertebrates are transmitted by blood-sucking ectoparasites within which they undergo a cyclical development. Whereas in the case of the *Glossina* mechanical transmission may occur it must play a subsidiary part. In *Glossina*, *Conorhinus* and leeches infection occurs through the mouthparts of the vector; in most *Glossinas* the flagellates are expelled from the salivary glands. In but a single instance (*T. inopinatum*) has it been claimed that the offspring of the vector (a leech) becomes hereditarily infected, and the statement awaits confirmation. In the rat-trypanosome there occurs a contaminative infection through the dejecta of the fleas or lice which attack a fresh host. Fleas and lice do not become hereditarily infected with *T. lewisi*. In dourine (*T. equiperdum*) direct infection from host to host in the act of coitus appears to be the rule, whereas it may occur exceptionally in other trypanosome infections. We are still ignorant as to the usual mode of infection of many trypanosomiases, including Surra and Mal de Caderas. In both cases the parasites (*T. evansi* and *T. equinum*) possess a wide range of pathogenicity and may produce chronic cases, so that there is every reason to believe, in my opinion, that reservoirs may play an important part in maintaining these diseases in nature.

Powers of adaptation shown by trypanosomes.

Investigations conducted during the last few years have shown that trypanosomes possess considerable power of adaptation to altered conditions in the host, such as may be brought about by the administration of drugs. Trypanosomes may acquire a great resistance to the effect of a drug. At times this acquired resistance is accompanied by changes in their morphology, at other times no such changes occur. In the case of *T. brucei*, which becomes resistant to pyronin and oxazine preparations, the acquired drug-resistance is accompanied by the disappearance of the blepharoplast. If these drugs are administered

to an animal harbouring the trypanosome in its blood it will be seen that 40 to 90% of the trypanosomes no longer show blepharoplasts after the expiration of 24 hours from the time the drug was administered. In this respect the oxazine preparation is the more powerful drug. If such trypanosomes are now passed through a series of 10 rats, each rat being in turn treated with the drug, it will be seen that the trypanosomes acquire a great resistance to the drug and that, with time, they no longer show blepharoplasts. If the trypanosomes have been subjected to the continuous effect of the drug for longer periods the blepharoplasts will not be reacquired even when the trypanosomes are passed through a series of 130 untreated animals. The oxazine preparation acts directly upon the blepharoplast. In trypanosomes which have reacquired their blepharoplasts a change of constitution has been brought about, for when they are subjected to the effects of arsenicals or tryparosan they again lose their blepharoplasts, this being contrary to what is observed in the normal flagellates.

In other cases no appreciable alteration in morphology accompanies the acquisition of drug-resistance. *Trypanosoma brucei* has been rendered resistant to parafuchsin (Francke and Roehl) and to atoxyl (Browning). In the latter case, this trypanosome has been found to remain drug-resistant during its passage through 140 untreated animals, this representing many generations of trypanosomes. There are, however, limitations to this resistance. Thus, Mesnil and Brimont found that *T. evansi*, rendered resistant to atoxyl to such a degree that the strain still remained resistant after passing through 110 untreated mice, when transferred *to another host*, the rat, immediately became susceptible to the drug whilst the rat served as its host. When the flagellate had passed through a series of 10 rats it was found, however, to be still atoxyl-resistant when returned to the body of the mouse. Similarly, atoxyl-resistant *T. equiperdum*, rendered resistant in the body of the donkey, has been passed successively through rats, guinea-pigs, rabbits and rats during a period of seven months and been found atoxyl-resistant upon being returned to the animal, the donkey, in which it was originally rendered resistant. From this we may conclude that atoxyl combines with a blood constituent to act upon the trypanosomes. When the flagellates have acquired resistance to atoxyl + mouse blood, they are still susceptible to atoxyl + rat blood or other blood than that of the mouse.

Of considerable interest, moreover, are certain observations of Gonder's (1911) upon arsenophenylglycin-resistant *T. lewisi*, wherein

it was found that the resistance was retained for upwards of three months in cultures, but *lost* after a sojourn in the rat louse by which, as we have seen, the parasite is transmitted. The drug resistance is lost by the flagellate after about 10 to 12 days in the louse, and this fact may be brought forward in support of the view that the louse is a definitive host of the trypanosome and that a sexual development of the parasite occurs in the louse—the sexual process, at a stroke, eliminating acquired characters previously maintained for thousands of asexual generations during the passage by inoculation from rat to rat.

This observation has a practical bearing in respect to human trypanosomiasis where arsenic-resisting strains of *T. gambiense* would be assumed, under certain conditions, as likely to be transmitted by *Glossina*. It will doubtless be found in this case, as with *T. lewisi*, that the passage of the flagellate through its vector renders it again susceptible to the action of trypanocidal substances.

In the time at my disposal I have only been able to dwell upon certain aspects of the subject of trypanosomiasis, and to bring out some of the many interesting problems which are being gradually solved by many workers.

The preventive measures directed against sleeping sickness have been dictated by experience gathered from research into the etiology of the disease, and, as our knowledge advances, so will our measures for combating the scourge have to be modified. The hope that the disease would be exterminated by resorting to the inspection and segregation of natives, to depopulation and the destruction of the habitats which are suitable breeding grounds of *Glossina palpalis*, have been but partially realised. The prolonged period of incubation and chronicity of the disease, coupled with the fact that fatal relapses have been known to occur after the lapse of years in apparently recovered cases render the disease very difficult to combat. A measure of immunity appears to be acquired after recovery, judged from animal experiment. Although treatment has given results which are encouraging, we are still far from the goal we wish to attain. The question of reservoirs has grown to be one of the greatest practical importance and the cry for the destruction of the game animals which serve as such is growing louder. It is for this reason that game destruction is at present being carried out over limited areas to see if it exerts any beneficial effect, although it appears very doubtful that this measure will prove useful, since it will drive the game elsewhere and so scatter the reservoirs into

other regions. It is, moreover, by no means certain that domesticated animals would not have to be likewise destroyed, for they, too, may act as reservoirs. In view of the wide range of pathogenicity possessed by *T. gambiense* there is always the possibility of animals serving as reservoirs which it will be practically impossible to exterminate. On the other hand there is hope that by reducing the number of reservoirs and Glossinas the chain of parasitism may be broken, as it may be in malaria by mosquito reduction. Judging from the observations on Glossinas captured in a wild state, but a very small number of these are infective; the percentage of such flies may be sufficiently reduced to greatly lessen the danger of infection through their agency. In the case of *G. palpalis*, which has a relatively stationary habitat in proximity to water, we have a comparatively easy problem to deal with as compared to *G. morsitans*. The latter fly, which conveys *T. rhodesiense*, is migratory, it ranges widely, and it resists dryness such as *G. palpalis* cannot withstand, and therefore to attack its habitats successfully appears practically impossible. A measure of protection will no doubt be afforded by putting the land, in the vicinity of human habitations, under suitable cultivation. We know that the main roads of travel are the most dangerous and that the old native measure in respect to Nagana of avoiding the fly belts by day is safely to be relied upon.

It is clear that the study of the biology of the carriers of trypanosomes has become one of great practical importance, and that we are gradually accumulating data upon which we can proceed in a rational manner to combat trypanosomiasis.

Note: Those desiring to consult the literature on Trypanosomiasis are referred to the *Sleeping Sickness Bulletin*. The Lecturer has purposely refrained from giving references so as not to burden the text.

NUTTALLIA UND *PIROPLASMA* BEI DER PIROPLASMOSE DER EINHUFER IN TRANSKAUKASIEN.

Von E. DSCHUNKOWSKY und T. LUHS.
(*Aus der Rinderpestserumstation, Surnobat.*)

(With Plates XIV and XV.)

Im Jahre 1908 haben wir auf dem IX. Internationalen Tierärztlichen Kongresse im Haag einen Bericht über die Piroplasmose der Pferde und Esel in Transkaukasien erstattet und etwas später eine kurze Bemerkung darüber in russischer Sprache publiziert. Nachher haben wir aber noch einige Fälle von Piroplasmose bei den Pferden beobachtet, wobei wir die Ansicht Nuttall's bestätigen konnten über die Existenz zweier selbstständiger Arten der Pferdepiroplasmose, welche durch zwei besondere Arten von Blutparasiten der Gattung *Nuttallia* und *Piroplasma* verursacht werden.

Im Nachstehenden geben wir eine Beschreibung aller von uns beobachteten Fälle der Piroplasmose der Einhufer, nebst neuen Zeichnungen, und stellen, im Einklang mit den neueren Ansichten, in Transkaukasien das Vorkommen mehrerer selbstständiger Arten dieser Piroplasmose fest.

I. Nuttallia.

Die unter den Einwohnern verbreitete Meinung, dass die Pferde in der Ebene an Malaria leiden, deutete auf das Vorkommen der Pferdepiroplasmose in Transkaukasien. Den ersten Fall dieser Piroplasmose in Transkaukasien beobachteten wir im Jahre 1905. In einem Dorfe von russischen Kolonisten im Tiflisser Kreise erkrankten importierte Pferde an einer schweren Krankheit, die mit dem Tode endigte. Die Bauern dachten es sei Malaria, wie bei den Menschen.

In diesem Dorfe konnten wir nur eine Sektion eines frischen Kadavers vornehmen. Nach der Beschreibung der Krankheit durch den Besitzer bestanden die Krankheitserscheinungen in Fieber, Depression, Verdauungsstörungen, welche sich in Diarrhöe äusserten und in Oedemen an verschiedenen Körperstellen, besonders an den Lippen. Das Tier war ungefähr zwei Wochen krank. Die Sektion ergab folgendes Bild: der Körper war ziemlich gut genährt. Die Conjunctiven waren blassgelb; die Ober- und Unterlippen ein wenig oedematös. Die subcutanen Gefässe zeigten sich injiziert, das Bindegewebe gelb gefärbt, die Muskulatur nicht verändert. Der ganze Drüsenapparat erschien angegriffen, die Lymphdrüsen vergrössert, an den Schnittflächen oedematös und hämorrhagisch. Am Ausgang des Magens befanden sich hämorrhagische Geschwüre von rundlicher Form, ungefähr drei Centimeter im Durchmesser. Der Dick- und Dünndarm erwies sich hyperämisch, und die Schleimhaut an einigen Stellen von verschieden grossen Hämorrhagien bedeckt. Die Milz war vergrössert und ihre Ränder abgerundet, die Pulpa jedoch ziemlich fest. Die Leber erschien auch vergrössert und von rotgelber Farbe. Die Nieren zeigten keine besonderen Veränderungen. In der Blase wurde ein wenig rotgefärbter Harn gefunden.

Bei der mikroskopischen Untersuchung von Blutausstrichpräparaten, welche nach Giemsa gefärbt waren, erwiesen sich etwa 1% aller Erythrozyten mit Parasiten besetzt, welche sich durch auffallende Grossen- und Formendifferenz auszeichneten. Was die letztere anbetrifft, so muss man als Grundtypus unserer Parasiten die Kugelform ansehen, weil sie die bei weitem häufigste ist und mehr als $\frac{3}{4}$ aller Formen ausmacht. Von den anderen Formen sind zu erwähnen: die ovale, die birnförmige und die amoeboide Form, welche alle, wie es scheint, aus der Grundform bei den verschiedenen Erscheinungen der Lebensbetätigung der Parasiten hervorgehen. Die Birnenform erinnert wohl an die gleiche Form des später zu beschreibenden *Piroplasma caballi*, ist aber viel einfacher in der Konstruktion, wie unten ausführlicher gesagt sein wird.

Was die amoeboide Form anbelangt, so ist sie bei unseren Parasiten eigentlich wenig ausgeprägt, da die Plasmafortsätze nur kurz oder kaum angedeutet sind und dabei der Körper der Parasiten sich auch wenig verändert. Dagegen glauben wir bei der Betrachtung gefärbter Blutausstriche der afrikanischen *Nuttallia equi* einen Unterschied von unseren Parasiten darin gefunden zu haben, dass die afrikanischen Parasiten besonders reich an amoeboiden Formen sind, welche hier

sozusagen den Haupttypus ausmachen, während die Kugelform dort weniger zahlreich vorhanden ist, als bei unseren Parasiten.

Von weiteren Parasitenformen muss die ziemlich häufig vorkommende kreuzförmige Teilungsform ganz besonders erwähnt werden, da sie ein charakteristisches Unterscheidungsmerkmal bei der Klassifikation der Gattung *Nuttallia* bildet, indem die Vermehrung der Parasiten dieser Gattung durch diese Form vor sich geht. Hierbei teilt sich der Kern der Mutterzelle in vier gleiche, kreuzförmig gelagerte Teile, welche sich mit Plasma umgeben und bald frei werden. (Pl. XIV, figs. 7, 8.) Die Kerne dieser jungen Parasiten sind rund und voluminös. Anfangs ist das Plasma dieser Formen wenig entwickelt und gleicht an Umfang dem Kern, oder übertrifft diesen nur um ein Weniges. Es färbt sich in einen gleichmässig dichten, blauen Ton, ohne Vakuolen und besonderer Verdichtung an der Peripherie.

Die jungen Parasiten haben gleich nach der Teilung meist eine runde oder leicht ovale Form. Ihr Kern liegt immer an der Peripherie und ist von keiner helleren Zone umgeben, wie bei den erwachsenen Formen. Häufig ist aber die Form der jungen Parasiten nagelförmig, wenn das Plasma sich an *einer* Seite des Kernes zuspitzt (Pl. XIV, fig. 13), oder spindelförmig, wenn diese Zuspitzung auch nach der entgegengesetzten Seite sich erstreckt (Pl. XIV, fig. 9, Pl. XV, fig. 8).

Was den Kern der *erwachsenen* Parasiten anbelangt, so ist derselbe fast immer einzeln, hat eine mehr oder weniger abgerundete Form und liegt gewöhnlich an der Peripherie, seltener in der Mitte des Parasiten. Nur schwer und meist garnicht gelingt es den Blepharoplasten zu erblicken, da er meist im Kern oder dicht bei demselben gelagert ist. Bisweilen ist der Kern in den kugelförmigen Parasiten die Peripherie entlang in Gestalt eines längeren oder kürzeren dünnen Bogens gelagert, wobei in demselben dunkler gefärbte, Körnchen zu sehen sind.

Das *Plasma* der erwachsenen Parasiten nimmt bei Giemsafärbung eine blaue Farbe an und zeigt oft eine netzartige Struktur. Gegen die Peripherie hin, verdichtet sich das Plasma und bildet einen deutlichen intensiver gefärbten Saum. Um den Kern herum, oder an der Seite des Kernes, welche dem Plasma zugewandt ist, bemerken wir fast immer eine heller färbbare Zone.

Wir können nicht umhin noch auf eine Eigentümlichkeit unserer Parasiten hinweisen, welche beim ersten Blick in das Mikroskop sofort auffällt: das ist die ungewöhnlich starke *Grössendifferenz* bei den einzelnen Formen. So schwankt der Durchmesser der kugelförmigen

Parasiten von 0·8 μ bis 2·8 μ; die Länge der birnförmigen Parasiten von 1·5 μ bis 4·0 μ, ihre Breite von 1·0 μ bis 1·5 μ.

Hierbei scheint es uns jedoch, dass diejenigen Parasiten, welche die *kleinsten*, ebenerwähnten Grössenverhältnisse aufweisen, keineswegs junge Formen sind, wie sie aus der Teilung hervorgehen, sondern schon erwachsene, reife Exemplare, mit allen Eigentümlichkeiten derselben. Dieser Umstand veranlasst uns zu der Vermutung, dass wir es in unseren Fällen der Pferdepiroplasmose nicht mit einer einzigen Art der *Nuttallia* zu tun haben, sondern mit zwei Arten, welche gemischt vorkommen, wobei die Parasiten der einen Art gross, die der anderen klein sind. Nur so, scheint es uns, kann man die auffallende Grössendifferenz erklären. Was dieses für Arten der Gattung *Nuttallia* sind?—entspricht unsere grössere Art der afrikanischen *Nuttallia equi*?—das sind Fragen, die wir augenblicklich noch nicht entscheiden können.

Es ist möglich, dass in Transkaukasien drei selbstständige Arten der Pferdepiroplasmose vorkommen und dass man überhaupt folgende Arten dieser Krankheit wird registrieren müssen:

1. *Nuttallia equi.*
2. Grosse *Nuttallia* in Transkaukasien.
3. Kleine *Nuttallia* in Transkaukasien.
4. *Piroplasma caballi.*

Nach diesen morphologischen und aetiologischen Betrachtungen wollen wir die weiteren von uns 1906 und 1907 beobachteten Fälle der Pferdepiroplasmose hier kurz anführen, wobei wir erwähnen müssen, dass auch in diesen Fällen die Parasiten zu der Gattung *Nuttallia* zu rechnen waren und mit den beim ersten Falle beschriebenen übereinstimmten. Eine Ausnahme bildet der weiter unten zu beschreibende Fall von *Piroplasma.*

Den *zweiten Fall* der Piroplasmose beobachteten wir bei einem Pferde, welches aus dem Nordkaukasus stammte und längere Zeit in der heissen Niederung des Kura-Flusses verbracht hatte. Es starb am 7. VIII. 1906. Angaben über die Erscheinungen der Krankheit fehlten. Wir konnten nur die Sektion machen, wobei wir eine allgemeine, zitronengelbe Färbung des Kadavers fanden. Das Blut war lackfarben und dünn. Die Leber hyperämisch, von gelber Farbe; die Milz ein wenig vergrössert; die Nieren blass, anämisch, ohne besondere Veränderungen; der Urin hell. Im Herzblute und in der Milz wurden spärliche kugelförmige *Nuttallia* gefunden.

Fall 3. Ein Pferd der örtlichen tatarischen Rasse, zu besonderen Versuchen für die Station angekauft. Es war ein abgemagertes Tier

mit Geschwüren am Widerrüst und an den Schultern. Einen Monat nach dem Ankauf starb das Tier am 29. x. 1906. Bei der Sektion des Kadavers entsprachen die pathologisch-anatomischen Veränderungen im Allgemeinen dem Fall 3. Im Milzblute wurden spärliche *Nuttallia* gefunden.

Fall 4 und 5 betreffen zwei ausbrackierte Artilleriepferde, welche ebenfalls von der Station zu Versuchszwecken gekauft waren. Beide Pferde stammten aus dem Nord-Kaukasus. Das eine starb am 13. viii. 1906, das andere am 17. xi. 1906. Das klinische Krankheitsbild dieser zwei Fälle gelang es uns leider auch nicht zu verfolgen, da die Pferde nicht auf der Station erkrankten. Die Sektion ergab das uns schon bekannte Bild. Die Blutuntersuchung zeigte Parasiten der Gattung *Nuttallia* im Blute der parenchymatösen Organe.

Fall 6. Im Juni 1907 wurde eines der ausbrackierten Artilleriepferde, aus derselben Partie wie Fall 4 und 5, auf einige Zeit in einem Dorfe am Ufer des Kura-Flusses im Elisabethpolschen Kreise eingestellt. Bald stellten sich hier bei dem Tier, nach den Aussagen des Wärters, Unlust und Mattigkeit ein, und es wurde Schüttelfrost bemerkt. Am 17. Juni 1907 untersuchten wir das Pferd und konstatierten grossen Kräfteverfall, Niedergeschlagenheit, und Appetitverlust; die Temperatur betrug 39·5° C. Die Schleimhäute des Maules und der Augen waren anämisch und zitronengelb gefärbt. Im venösen Blute fanden wir etwa 5% aller Erythrozyten mit Parasiten der Gattung *Nuttallia* besetzt. Die Zahl der Parasiten wuchs so schnell, das am 19. vi. 1907 schon 30 % bis 40 % aller Erythrozyten damit infiziert waren. Am 19. vi. starb das Tier. Die Sektion ergab dasselbe Bild, wie in den vorhergehenden Fällen. Blutiger Harn wurde nicht gefunden.

Wir konnten also im Jahre 1905 bis 1907 im ganzen 6 Fälle von Pferdepiroplasmose registrieren, wobei 5 Fälle importierte Pferde und 1 Fall ein einheimisches Pferd betraf. In 5 Fällen hatten wir es mit akuter und subakuter Erkrankung und nur in 1 Falle mit chronischer Erkrankung zu tun.

Leider gelang es uns nicht das volle klinische Krankheitsbild zu verfolgen, obgleich es im allgemeinen in Mattigkeit, Appetitverlust und Temperatursteigerung sich äussert. Das Auftreten von Blutharnen kann nicht zu den beständigen Symptomen gezählt werden.

II. Piroplasma.

Ein Fall von Mischinfektion von Piroplasma *mit* Nuttallia.

Dieser Fall betrifft ein ausbrackiertes Artilleriepferd aus derselben Partie, in welcher schon die beschriebenen Fälle 4, 5 und 6 von Piroplasmose vorgekommen waren. Es war ein Pferd der Kabardinischen Rasse aus dem Nordkaukasus. Bis 1910 befand es sich in gutem Ernährungs- und Gesundheitszustande. In den ersten Tagen des Juni-Monates 1910 erkrankte das Pferd nach einem langen, scharfen Ritte während grosser Hitze und bekam Blutsturz aus der Nase.

Am 5. Juni wurde das Tier auf die Station gebracht. Es war apathisch und ohne Appetit.

6. Juni. Temperatur: Morgens 38·2°, Abends 39·1° C.

7. Juni. Temperatur: Morgens 39·0°, Abends 40·1° C. Das Pferd begann zu fiebern. Bei der Blutuntersuchung wurden spärliche *Piroplasma* von typischer, grosser Birnenform zu zweien, seltener einzeln in den Erythrozyten gefunden. Ausserdem wurden auch amoeboide Formen mit kurzen Plasmafortsätzen beobachtet. Die Erythrozyten sind normal gross und färben sich gut. Die Zahl der Leukozyten, besonders der mehrkernigen, ist bedeutend vermehrt.

8. Juni. Temperatur: Morgens 41·0, Abends 41·2° C. Das Tier atmet schwer und frisst nichts. Im Blute ist die Zahl der Parasiten etwas grösser, als 7. VI. Sie haben fast ausschliesslich die grosse Birnenform.

9. Juni. Temperatur: Morgens 41·0, Abends 41·2° C. Die Zahl der Parasiten ist vergrössert; etwa 1—2 % aller Erythrozyten sind mit ihnen besetzt. Meist sind sie birnenförmig, seltener oval. Man sieht auch amoeboide Formen mit langen Fortsätzen. Bei sorgfältiger mikroskopischer Untersuchung wurde nebst einer gewissen Anzahl runder, kugeliger Formen in einem Praeparat eine kreuzförmige Teilungsform eines Parasiten in 4 neue Parasiten gefunden. Da diese Teilungsform für Parasiten aus der Gattung *Nuttallia* charakteristisch ist, so haben wir es hier augenscheinlich mit einer Doppelinfektion zu tun: mit Parasiten aus der Gattung *Piroplasma* und *Nuttallia*. Zu dieser letzteren Gattung sind auch die verschieden grossen kugelförmigen Parasiten zu zählen, welche neben den grossen birnförmigen auftreten.

10. Juni. Temperatur: Morgens 41·1°, Abends 40·3° C. Die Zahl der Parasiten ist wie am 9. VI. Man beobachtet beim Tier Zittern des Körpers.

11. Juni. Temperatur: Morgens 41·1°, Abends 41·3° C. Die Parasiten vermehren sich nur langsam und besetzen etwa 2—3 % aller Erythrozyten. Diese letzteren zeigen keine besonderen Veränderungen, weder qualitativ noch quantitativ. Bei einigen birnförmigen und ovalen Parasiten sieht man den Kern in 2—4 Körnchen zerfallen.

12. Juni. Temperatur: Morgens 39·9°, Abends 39·7° C. Die Zahl der Parasiten ist ein wenig vermindert. Vorherrschend sind einkernige ovale Formen.

13. Juni. Temperatur: Morgens 39·7°, Abends 37·0° C. Das Tier starb am Abend.

Die Sektion ergab nichts Charakteristisches für die Piroplasmose, ausser einer sehr starken, 5 bis 6-maligen Vergrösserung der Milz welche von Aussen bläulich grau, auf dem Schnitte dunkelrot gefärbt und eine ziemlich feste, derbe Konsistenz aufwies. Ihre Ränder waren abgerundet. Der Harn war nicht rot gefärbt. Ebensowenig wurde auch eine Gelbfärbung der Schleimhäute, der serösen Häute und anderer Organe des Kadavers gefunden. Dieses erklärt sich wohl durch den sehr schnellen Verlauf der Krankheit, der so kurz war, dass das Blut sich in der für die Piroplasmose charakteristischen Weise nicht verändern konnte. Ferner wurden auch Hämorrhagien nirgends beobachtet.

Was die *Morphologie* der beobachteten Parasiten anbelangt, so haben wir es hier, wie schon gesagt, mit zwei Arten aus der Gattung *Piroplasma* und *Nuttallia* zu tun.

Piroplasma. Diese Parasiten haben eine typische, grosse, birnenförmige Gestalt und erinnern an die grossen, typischen Birnen der Rinderpiroplasmose. Sie befinden sich seltener einzeln, meist zu zweien, aber nicht zahlreicher in einem Erythrozyten. In letzterem Falle sind sie fast immer an den zugespitzten Enden miteinander verbunden. An der Verbindungsstelle sieht man bisweilen eine kleines rundes Körnchen, welches die Chromatinfärbung annimmt. Das dicke Ende der Birnen ist stets abgerundet. Zuweilen liegen die birnförmigen Parasiten in einem Erythrozyten gesondert, wobei ihre spitzen Enden in die entgegengesetzte Richtung zeigen. Unter den Doppelbirnen trifft man nicht selten solche, bei welchen der eine Parasit die normale Form hat, während der andere bei gleicher Länge um die Hälfte dünner ist (Pl. XIV, figs. 30, 31). Hierbei ist das Volumen des Kernes dasselbe, wie bei dem normalen Parasiten; nur ist das Plasma reduziert, infolgewessen der Parasit fast ganz die Kernfärbung annimmt[1].

Die Grössenverhältnisse des beobachteten Piroplasmen sind folgende:

Die Länge der birnförmigen Parasiten schwankt zwischen $2{\cdot}4\,\mu$ und $3{\cdot}7\,\mu$: ihre grösste Breite von $1{\cdot}0\,\mu$ bis $1{\cdot}5\,\mu$. Man sieht zuweilen besonders schlanke Birnen, welche bei einer Länge von $3{\cdot}7\,\mu$ eine Breite von nur $1{\cdot}3\,\mu$ aufweisen.

Fast bei allen birnförmigen Parasiten, kann man in Praeparaten, die nach Giemsa gefärbt sind, Kern und Blepharoplast unterscheiden.

[1] Diese Erscheinung ist durch die flache Form der intrakorpuskulären birnenförmigen Parasiten bedingt, die Schmalen sind einfach solche welche in Seitenansicht vorliegen. Davon kann man sich dadurch überzeugen, dass man lebende Parasiten untersucht, wobei Rotationsbewegungen zuweilen vorkommen. G. H. F. N.

Letzterer ist sehr kompakt und färbt sich viel intensiver und schneller als der Kern in einen dunkel karminroten Ton. Meist liegt er gesondert vom Kern in dem spitzen Ende der Birne oder an dessen Peripherie. Zuweilen liegt aber der Blepharoplast dicht neben dem Kern, oder sogar im Kerne selbst.

Als eine auffallende Erscheinung muss man bei den birnförmigen oder ovalen Parasiten die Bildung seitlicher knospenartiger Auswüchse bezeichnen, welche augenscheinlich in enger Beziehung zu der Teilung und Neubildung der Parasiten stehen[1]. Diese knospenartigen Fortsätze bilden sich immer paarweise und fast stets an ein und derselben Seite des Mutterparasiten, seltener an beiden Seiten (Pl. XIV, figs. 25–29). Sie haben die Gestalt von kleinen Birnen und sind mit ihrem spitzen Ende mit der Mutterzelle verbunden. Sie entspringen beide zuweilen aus einer Stelle (Figs. 26, 28), zuweilen jedoch etwas weiter auseinander (Figs. 25, 29). Nach ihrem Freiwerden von der Mutterzelle bilden die Knospen, augenscheinlich, im ersteren Falle Doppelbirnen, im letzteren Falle dagegen neue einzelne Birnen. In den knospenartigen Auswüchsen kann man immer die charakteristischen Bestandteile des Parasiten, Kern und Blepharoplast, erkennen. Was das Schicksal der Mutterzelle anbelangt, so kann man in derselben am Anfang der Knospenbildung auch eine gewisse Menge Kernsubstanz wahrnehmen, welche jedoch mit dem Wachstum der neuen Birnen allmählich schwindet, wobei auch das Plasma immer blasser wird und zuletzt nicht mehr von der Farbe des Erythrozyten zu unterscheiden ist: man sieht nur noch die bläulichen Konturen der Mutterzelle. Die Länge solcher neuer Birnen erreicht 2·0 μ, die Breite 1·0 μ. In den birnförmigen Parasiten sieht man zuweilen eine Teilung des Kernes in eine grössere oder kleinere Anzahl Körnchen, welche entweder drinnen an der Peripherie anliegen (Figs. 22, 34) oder im ganzen Parasitenleibe verteilt sind (Fig. 34).

Neben den Birnen liessen sich beim Leben des Tieres auch *amoeboide* Formen beobachten, die aber ganz anders aussehen, als die oben erwähnten knospenbildenden Parasiten. Bei der amoeboiden Form zieht sich der unregelmässig geformte Körper in 2–3 dünne, zugespitzte, oder an den Enden verdickte Fortsätze oder Pseudopodien aus, welche fast immer Kernsubstanz enthalten, entweder nur an ihrem

[1] Diese sind die bei allen echten *Piroplasma* vorkommenden Teilungsformen welche zuerst von Nuttall und Graham-Smith vor Jahren bei lebenden *P. canis* als solche erkannt wurden. Das *ganze* Protoplasma geht in Teilung über, es überbleibt also kein Restkörper wie die Autoren zu glauben scheinen. G. H. F. N.

Grunde (Fig. 23) oder in ihrer ganzen Länge: hierbei färben sich die Fortsätze dann in einen karminroten Ton (Fig. 24).

Durch Abrundung des spitzen Endes der birnförmigen Parasiten entstehen ovale Formen, welche dann von ebensolchen Formen der *Nuttallia* nur schwer zu unterscheiden sind (Fig. 19).

Bis jetzt haben wir Parasiten aus dem Venenblute des lebenden Tieres betrachtet und übergehen nun zu deren Untersuchung im Kadaver.

Fünf Stunden nach dem Tode wurden bei der Sektion des Pferdes in den inneren Organen ausschliesslich abgerundete Parasitenformen beobachtet.

So waren im *Knochenmark* etwa 5 % aller Erythrozyten mit eckigen und abgerundeten Parasiten besetzt, deren Ränder meist leicht ausgezackt waren. Die Parasiten liegen hier einzeln oder zu zweien, seltener zu dreien in einem Erythrozyten. Ihre Kerne sind zusammengeschrumpft und liegen meist an der Peripherie. Der Blepharoplast ist nur schwer vom Kern zu trennen. Liegt der Kern an der Peripherie, so sieht man oft eine hellere Zone, die ihn vom Plasma abgrenzt.

Das *Plasma* der Parasiten färbt sich dicht blau und zeigt oft eine ungleichmässige, gekörnelte Struktur. Die Konturen der Parasiten sind nicht dichter blau gefärbt, als das Plasma, und heben sich deshalb fast gar nicht ab vom übrigen Plasma, im Gegensatz zu *Nuttallia*, wo die Konturen besser markiert sind. Der Durchmesser der grössten abgerundeten Parasiten aus dem Knochenmark beträgt 2·0 μ, der mittleren 1·4 μ, der kleinsten 0·7 μ bis 1·0 μ.

Zufolge eingetretener Degeneration ist es unter den Parasiten des Knochenmarkes schwer zu unterscheiden, welche Formen zur Gattung *Piroplasma*, welche zur *Nuttallia* gehören. Zu den letzteren sind mit Gewissheit nur diejenigen Parasiten zu zählen, welche sich zu dreien in einem Erythrozyten befinden.

In den *Nieren* nehmen die Parasiten etwa 1 % aller Erythrozyten ein und sind meist einzeln. Ihrer Form nach ähneln sie denen aus dem Knochenmark. Ihre Grösse schwankt zwischen 0·7 μ und 1·5 μ. In der *Milz* sind die Parasiten auch nur spärlich vorhanden und gleichen denen in den Nieren. In der Milz, wurden frei im Blutplasma spärliche, kreuzförmige Teilungsformen der *Nuttallia* gefunden.

Zum Schluss unserer Betrachtung der Erreger der Pferdepiroplasmose möchten wir noch auf einige unterscheidende Merkmale in der Birnenform bei *Nuttallia* und *Piroplasma* hinweisen.

Bei der *Nuttallia* kommt die Birnenform im Vergleich zu der Kugelform nur selten vor und ist mehr eine zufällige Erscheinung. Bei *Piroplasma* dagegen ist die Birnenform die vorherrschende und bildet ein Charakteristikum dieser Gattung. Bei *Nuttallia* kommt die Birnenform meist einzeln in den Erythrozyten vor; sind zwei Birnen in einem Erythrozyten, so sind sie nicht miteinander an den dünnen Enden verbunden. Bei *Piroplasma* kommen meist Doppelbirnen vor und sind miteinander verbunden.

Bei *Nuttallia* sind Kern und Blepharoplast fast immer zusammen, während bei der Birnenform des *Piroplasma* diese beiden Gebilde gut zu unterscheiden sind.

Ferner ist der Kern der *Nuttallia* abgerundet und meist in der Einzahl vorhanden. Bei *Piroplasma* ist der Kern länglich, oft mehrteilig.

Die Struktur der Birnenform der *Nuttallia* ist einfach, glatt, die des *Piroplasma* in gefärbten Präparaten mehr kompliziert, "bunt"— infolge der zahlreicheren Vakuolen, der körnigen, oft maschigen Struktur des Plasmas und der Mehrteiligkeit des Kernes.

Es bleibt uns noch übrig, 5 Fälle von Pferdepiroplasmose zu erwähnen, welche wir im Jahre 1910 und 1911 beobachtet haben. Diese betrafen (meist) teils einheimische, teils importierte Pferde (das eine Pferde war ein Ardenner). In allen diesen Fällen, welche tötlich verliefen, konnten wir im Blute der Tiere Parasiten aus der Gattung *Nuttallia* konstatieren.

Ziehen wir zum Schluss noch einmal alle obenerwähnten Fälle in Betracht, so sehen wir, dass in Transkaukasien die Piroplasmose der Pferde hauptsächlich durch Parasiten der Gattung *Nuttallia* bedingt wird, da von 12 Fällen dieser Krankheit nur in einem Falle Parasiten aus der Gattung *Piroplasma* gefunden wurden, und auch dann in Komplikation mit *Nuttallia*.

Piroplasmose des Maultieres.

Im Jahre 1906 konnten wir auch *Nuttallia* im Blute eines Maultieres konstatieren. Man brachte uns zur Untersuchung ein Maultier, welches mehrere eiternde Wunden und Abscesse an verschiedenen Körperstellen hatte. Temperaturerhöhung wurde nicht bemerkt und ausser starker Abmagerung wurden keine krankhaften Symptome beobachtet.

Bei der Untersuchung des Blutes aus der Drosselvene gelang es einzelne spärliche Parasiten zu finden, welche in Form und Grösse den bei den Pferden beobachteten Parasiten entsprachen, wobei aber runde

Formen vorherrschten. Am 30. VIII. wurden mit dem Blute dieses Maultieres 1 Pferd und zwei Esel infiziert. Dem Pferde wurden 800,0 c.cm. defibrinierten Blutes eingespritzt. Weder Temperaturerhöhung noch andere Symptome wurden bei ihm beobachtet, auch gelang es nicht im Blute Parasiten zu finden. Beim ersten Esel, welchem 500,0 c.cm. desselben Materials eingespritzt wurden, konnten wir auch keine Reaction bemerken. Der zweite, junge Esel, welcher 100,0 c.cm. Blut subcutan erhielt, zeigte auch ausser starker Abmagerung keine Krankheitserscheinungen. Am 13ten Tage nach der Infektion fiel er jedoch plötzlich, wobei in seinem Blute Parasiten gefunden wurden. Die Sektion ergab nichts ausser Anämie und einer geringen Vergrösserung der Milz.

Den Tod dieses Tieres der künstlichen Infektion zuzuschreiben getrauen wir uns jedoch nicht, da in diesem Falle chronische Krankheitserscheinungen vorlagen, weshalb es möglich ist, dass die Infektion den tötlichen Ausgang bei dem Tier bloss beschleunigte. Das Tier muss schon früher an Piroplasmose krank gewesen sein, was uns jedoch beim Leben festzustellen nicht gelang.

Piroplasmose der Esel.

Zwecks Feststellung der Ausbreitung der Piroplasmose unter den Eseln erwarben wir im August und September 1906 in verschiedenen Gegenden, hauptsächlich im Gouv. Elisabethpol, 18 völlig kraftlose, zur Arbeit untaugliche Esel. Es waren abgemagerte Tiere, welche kaum die Füsse rührten und sich im Zustande völliger Abzehrung befanden. Die Tiere hatten während der ganzen Zeit der Beobachtungen normale, einige sogar subnormale Temperatur.

Das weitere Schicksal dieser Tiere ist wie folgt:

Esel No. 1. Blieb am Leben. Bei ihm wurde vom 15. IX. bis 26. X. Temperatur gemessen, welche immer normal war. Im Venenblute wurden spärliche Parasiten gefunden.

Esel No. 2. Wurde am 31. VIII. aus dem Dorfe Tschaikend des Elisabethpolschen Kreises gebracht. Die Temperatur wurde vom 1. IX. bis zum 15. IX. gemessen; sie war normal, des Morgens sogar subnormal. Das Tier ging an Erschöpfung zu Grunde am 16. IX.

Bei Lebzeiten des Tieres konnten wir im Blute aus der vena jugularis Parasiten nachweisen, und nach dem Tode wurden sie auch in der Milz gefunden.

Esel No. 3. Ein sehr stark erschöpftes Tier. Es ging an Kachexie ein am 10. XI. Die Temperatur erwies sich während der Beobachtungszeit als normal und unternormal. Parasiten im Blute nachzuweisen gelang nicht.

Esel No. 4. Die Temperatur war die ganze Zeit—bis zum 20. XII.—normal. Parasiten wurden nicht gefunden.

Esel No. 5. Wurde am 20. IX. getötet. Spärliche Parasiten.

Esel No. 6. Ging an Erschöpfung ein am 3. XI. Parasiten wurden nicht beobachtet.

Esel No. 7. Ging an Erschöpfung ein. Parasiten konnten nicht gefunden werden.

Esel No. 8. Ging an Erschöpfung ein am 26. XI. Parasiten wurden nicht gefunden.

Esel No. 9. Ging an Erschöpfung ein am 16. IX. Im Venenblute, in der Milz und anderen parenchymatösen Organen wurden etwa 1 % Erythrozyten mit Parasiten besetzt gefunden. Mit dem von diesem gewonnenen, defibrinierten Blute wurde ein Pferd subkutan geimpft, wobei ihm 180,0 c.cm. eingeführt wurden. Das Pferd blieb in der Folge ganz gesund und zeigte auch keine Temperatursteigerung. In seinem Blute wurden auch keine Parasiten gefunden.

Esel No. 10. Blieb am Leben. Im Blute wurden keine Parasiten entdeckt.

Esel No. 11. Ging am 3. X. an Erschöpfung ein. Im Blute wurden sehr spärliche Parasiten gefunden.

Esel No. 12. Blieb am Leben. Keine Parasiten.

Esel No. 13. Ging am 24. IX. an Kachexie ein. Die Untersuchung des Blutes auf Parasiten fiel negativ aus.

Esel No. 14. Ging am 17. IX. an Kachexie ein. Im Blute spärliche Parasiten.

Esel No. 15. Ging am 22. IX. an Kachexie ein. Spärliche Parasiten im Blute.

Esel No. 16. Ging am 5. X. an Kachexie ein. Spärliche Parasiten.

Esel No. 17. Ging am 11. IX. an Kachexie ein. Parasiten wurden nicht gefunden.

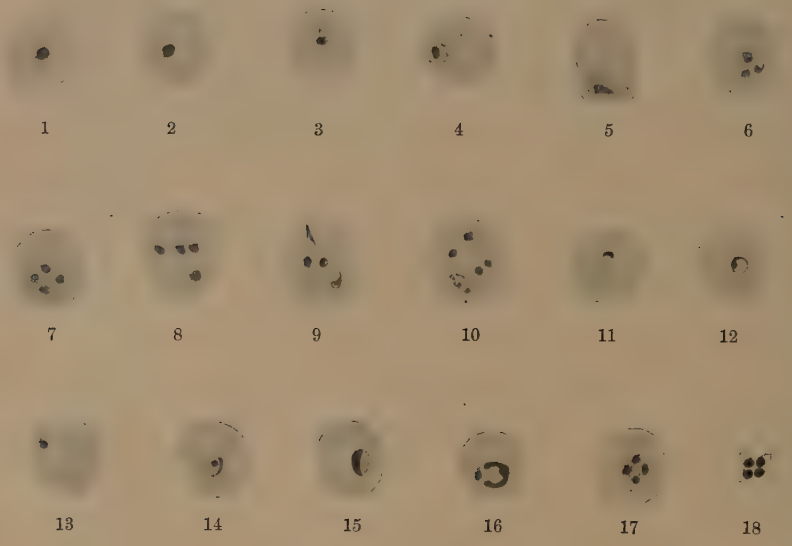
Esel No. 18. Blieb am Leben. Keine Parasiten.

So haben wir von 18 Eseln bei 8 resp. bei 44,4 % Parasiten gefunden. Im Verlaufe von 1–2 Monaten gingen von den 18 Eseln 11 resp. 61,1 % ein, wobei 4 derselben im Blute Parasiten aufwiesen. Die Sektion ergab nichts ausser Anämie. Man konnte auch eine bemerkbare Milzschwellung nicht konstatieren.

Vier Esel blieben mit Parasiten im Blute am Leben.

Die hier angeführten Tatsachen sprechen für eine weite Verbreitung der Piroplasmose unter den Eseln in Transkaukasien und wahrscheinlich auch in den Nachbarländern, Persien und der Turkei, da es von unseren Tieren bekannt war, dass sie lange Zeit in den benachbarten persischen Provinzen gearbeitet hatten. Vermutlich ist die Mehrzahl der erschöpften, mageren Esel, wie man sie so oft auf den Wegen und Markten sieht, Opfer der Piroplasmose.

Die Frage nach der Identität der von uns bei der Piroplasmose der Pferde in Transkaukasien beobachteten Parasiten mit den beschriebenen Arten *Nuttallia equi* und *Piroplasma caballi* kann, wie wir schon oben gesagt haben, augenblicklich noch nicht mit Sicherheit entschieden



Nuttallia equi (Laveran).

Piroplasma caballi Nuttall.

werden. Ein Vergleich von gefärbten Ausstrichpräparaten von Parasiten verschiedener Herkunft und Arten, giebt hier allein keinen sicheren Aufschluss. Dasselbe gilt auch von der Identifikation der Eselpiroplasmose in Transkaukasien mit *Nuttallia equi*. Eher scheint uns das Gegenteil der Fall zu sein. Und wir glauben deshalb nicht fehl zu gehen, wenn wir, in Anbetracht der immer weiter fortschreitenden Teilung der Pferdepiroplasmose in *Nuttallia* und *Piroplasma*, den Parasiten der Eselpiroplasmose in Transkaukasien von der Pferdepiroplasmose zeitweilig trennen und als besondere Art aufstellen, welche wir *Nuttallia asini* (n. sp.) zu benennen vorschlagen.

ERKLÄRUNG ZU TAFEL XIV UND XV.

TAFEL XIV.

Nuttallia equi (Laveran).

Circa × 3000. Giemsafärbung. Figs. 1–17 aus dem Venenblute, Fig. 18 aus der Milz des Pferdes.

Figs. 1 and 2. Einzelne junge Parasiten mit grossem Kern, in frische Erythrozyten eingewandert.
Figs. 3 and 4. Amoebenartige Parasiten, ohne ausgesprochene Fortsätze.
Fig. 5. Einzelner birnenförmiger Parasit mit einem Kern.
Fig. 6. Einzelner ovaler dreikerniger Parasit.
Fig. 7. Kreuzförmige Teilungsfigur: es bilden sich 4 neue Parasiten mit Kernen.
Fig. 8. Vier junge Parasiten nach der Teilung.
Fig. 9. Erythrozyt mit 4 Parasiten.
Fig. 10. Erythrozyt mit 5 Parasiten, davon 4 junge mit runden Kernen; der fünfte von ovaler Form, mit unregelmässiger Kern.
Figs. 11–13. Sehr kleine Formen.
Fig. 14. Ausgewachsener Parasit.
Fig. 15. Parasit mit voluminösem Kern und dunkelblauem Plasma.
Fig. 16. Parasit mit pferdeeisenförmige Chromatinmasse (herangehende Teilungsform nach Ansicht Nuttalls).
Fig. 17. Kreuzförmige Teilungsfigur.
Fig. 18. Dasselbe aus dem Blutplasma der Milz.

Piroplasma caballi Nuttall.

Circa × 3000. Giemsafärbung. Infizirte Erythrozyten aus dem Venenblute des Pferdes.

Fig. 19. Ovaler Parasit, veilleicht *Nuttallia*.
Figs. 20, 22. Einzelne birnenförmige Parasiten.
Figs. 21, 23, 24. Einzelne amoeboide Parasiten.
Figs. 25–29. Teilungsformen.
Figs. 30–35. Typische Doppelparasiten, zusammenhängend resp. getrennt.
Fig. 36. Ein aus dem Erythrozyt herausschlüpfender Parasit.

TAFEL XV.

Nuttallia beim Maultier.

Parasiten aus dem Venenblute eines chronisch erkrankten Maultieres.

Figs. 1, 2. Birnenförmige Parasiten.
Figs. 3, 4. Unregelmässige Formen.
Figs. 5, 6. Kugelförmige Parasiten.

Nuttallia asini n. sp.

Parasiten im Blute verschiedener Esel.

Figs. 7, 8. Einzelne Parasiten mit einem Kern.
Figs. 9, 10. Einzelne Parasiten mit zwei Kernen.
Figs. 11–14. Erythrozyten welche zwei Parasiten enthalten.
Fig. 15. Dreikerniger Parasit.
Fig. 16. Kreuzförmige kürzlich entstandene Teilungsform.
Fig. 17. Erythrozyt mit 3 Parasiten.
Fig. 18. Erythrozyt mit 2 Theilungsformen, das eine auffallend klein.

1 2 3

4 5 6

Nuttallia in Mules.

7 8 9 10

11 12 13 14

15 16 17 18

Nuttallia asini n.sp. Dschunkowsky and Luhs.

INDEX OF AUTHORS

INDEX OF SUBJECTS

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS